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AQUATIC FIELD SURVEYS AT IOWA, RADFORD AND JOLIET ARMY AMMUNITION PLANTS

FINAL REPORT

VOLUME III - MICROBIOLOGICAL INVESTIGATIONS, IOWA AND JOLIET ARMY AMMUNITION PLANTS

(6)

AQUATIC FIELD SURVEYS AT IOWA, RADFORD, AND JOLIET ARMY AMMUNITION PLANTS.

~~ANNUAL REPORT~~

VOLUME III. MICROBIOLOGICAL INVESTIGATIONS, IOWA AND JOLIET ARMY AMMUNITION PLANTS.

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SUMMARY

This report describes the methodology and results of microbiological field and laboratory studies conducted at the Iowa and Joliet Army Ammunition Plants during 1975. The purpose of this study was to establish the impact of microorganisms in determining the environmental fate of TNT wastewater in receiving streams. An attempt was made to define threshold toxicity levels and relative rates of TNT transformation in streams receiving low volume waste discharges (IAAP) versus streams receiving high volume waste discharges (JAAP).

The IAAP is located ten miles west of Burlington, Iowa. The plant consists of approximately 20,000 acres of which about 7,000 acres are leased for agriculture, 7,500 acres are forested, and the remaining acreage being used for administrative and industrial operations.

The major receiving stream of interest for these studies was Brush Creek which flows through the east central portion of the plant and ultimately into the Skunk River. It drains a watershed of about 6,300 acres of which 5,300 acres are plant property and except during periods of rainfall, its flow consists mainly of treated industrial waste discharges and effluent from the main sewage treatment plant which serves the IAAP facilites.

Five stations in upper Brush Creek were chosen for the field survey conducted at the IAAP in June 1975 as this section of the stream receives the majority of the munitions process water currently being discharged at this installation. One station was located upstream of any known discharge, while the remaining four stations were located below known effluents from the various process and loading facilities.

Sampling stations at JAAP were located on Grant Creek and the open wastewater channel known as "TNT Ditch". The concentrations of munition compounds discharged into the TNT Ditch and lower Grant Creek are higher

than those measured at the IAAP, offering the opportunity to study the assimilative capacity of microorganisms under high doses. Two stations were selected in Grant Creek for the May-June 1975 survey. One station was located upstream from the tetryl area and served as a control for the JAAP survey. The second station was located 10 meters downstream from the confluence of the TNT Ditch and Grant Creek, in a zone where mixing is reasonably complete. Sampling stations in the TNT Ditch consisted of an area 10 meters downstream from the outfall of the Red Water Disposal Facility and the second station approximately 2 meters upstream from the confluence of the TNT Ditch and Grant Creek. An open "red water" pond located at the northwest corner of the Red Water Disposal Facility was chosen as a sampling station because the pond received especially high levels of munitions compounds and was subjected to a maximum amount of sunlight.

Water samples from Brush and Spring Creeks at IAAP were collected on a grab basis once per day for five days during both the June and October 1975 surveys at this installation. Replicate sediment cores were also taken at each station during these surveys. Water and sediment samples were collected one time from each station at JAAP during the first week of June 1975. Grab samples were taken of the aqueous phase, while sediments were collected using coring tubes. Aqueous and sediment samples from IAAP and JAAP were analyzed for major mineral constituents, nutrients, trace metals and specific munitions compounds relating to the production and handling of trinitrotoluene.

The microbiological survey at the IAAP and the JAAP consisted of an assessment of the following parameters; bacterial enumeration, microbial inhibition study, benthic dissolved oxygen uptake, dehydrogenase activity and ATP activity. Laboratory investigations included; microbial degradation studies with indigeneous sediment populations, monitoring TNT degradation and toxicity in anaerobic systems, and a bacteriological toxicity study of munitions-related compounds.

The planktonic and benthic bacterial density at the IAAP and JAAP were within reported ranges for stream water (10^5-10^6 cells/milliliter) and sediment (10^7-10^8 cells/gram dry weight). Concentrations of trinitrotoluene in the aqueous phase approaching 1.5 ppm at the JAAP did not affect the oxygen utilization of an unacclimated microbiological seed.

Sediment microbiological activity at stations receiving munition wastes, as determined by oxygen uptake rates, dehydrogenase and ATP, did not vary appreciably from the control station. Sediment TNT concentrations approached 338 mg/kg and 44,200 mg/kg at the JAAP.

The microbiological degradation studies with indigeneous sediment populations revealed that the transformation/degradation of TNT appears to be a co-metabolic process which requires an added carbon source. Total mineralization of TNT was not observed as the transformation studies revealed persistant end products. The rate of transformation of TNT by microorganisms was shown to be a fairly rapid process and appears to be concentration dependent. Acclimated and unacclimated populations from the IAAP and the JAAP transformed approximately 70-90 percent of a 10 ppm and 100 ppm concentration of TNT within 3 to 5 days. An increase in cell density was observed with cultures grown in the presence of 100 ppm TNT versus 10 ppm TNT.

The anaerobic degradation studies revealed that initially concentrations of 100 ppm TNT inhibited methane production, but concentrations of TNT eliciting a toxic response increased to greater than 200 ppm as the acclimation period increased.

The major transformation products observed in the microbial degradation studies were tentatively identified as; 4-hydroxylamino 2,6-dinitrotoluene, 2-hydroxylamino 4,6-dinitrotoluene, 4-amino 2,6-dinitrotoluene, 2-amino 4,6-dinitrotoluene, 2,2',6,6'tetranitro-4,4'azoxytoluene, and the 2,4-diamino-6 mononitrotoluene. The hydroxylamino-DNT transformation products were qunatified in the field samples with concentrations

as high as 154 mg/kg being found at JAAP. These transformation products are formed in chemical and biochemcial reduction processes, through a variety of mechansisms.

Researchers involved in studying the photolysis of TNT have identified a number of transformation products, most of which differ from the products observed in our microbial degradation studies. Chemical reduction studies of TNT have produced compounds which are identical to the microbiological transformation products identified by various authors, indicating a similarity in the chemical and biochemical reduction process. Generally, chemical reduction of polynitro aromatics under acidic conditions produces amines, while reduction under neutral conditions produces hydroxylamines, and reduction under basic conditions produces dimers such as hydrazo, azo, and azoxy compounds. The effect of pH on TNT reduction underscores the importance of hydrolysis reactions in the transformation of this munitions compound. The relative importance of each of these three processes (photolysis, reduction, and hydrolysis) depends, of course, on the conditions present at the time of transformation.

Appreciable evidence exists that all three processes described are operational, to one extent or another, in the environment. In the aqueous phase of natural systems where natural sunlight is available, photolysis and soil systems, where TNT has been deposited through sorbtion processes, photo-energized reactions are severely limited. In these environments, chemical and biochemical reduction are the major steps in the primary transformation of nitrobodies like TNT. Through direct and indirect action, the microbiological community plays an important role in the environmental fate of TNT. Cell density and microbial activity studies coupled with laboratory degradation experiments have shown the existence of an established microbial population with the capabilities to rapidly transform TNT depending on concentration and nutrient availability. It appears that aqueous and sediment microbial communities inherently contain organisms with the ability to transform TNT as an extensive acclimation period was not requied. Indigenous populations isolated from control stations and stations with varying concentrations of TNT exhibited similar transformation rates.

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ABSTRACT

The role of microorganisms in controlling the environmental fate of alpha TNT was examined at IAAP and JAAP. Intensive surveys at these locations coupled with laboratory studies attempted to determine threshold toxicity levels of munitions waste discharges to indigenous microbiological populations. Photochemical transformation products were quantified in an attempt to further delineate the environmental fate of TNT.

SECTION I
CONCLUSIONS

1. The concentrations of munitions-related compounds in streams at the IAAP are generally low, and thus provide conditions suitable for the study of chronic exposure to such materials.

2. The concentrations of munitions-related compounds in TNT Ditch and lower Grant Creek at JAAP are relatively high, and thus provide conditions suitable for the study of acute exposure to these materials.

3. The environmental fate of 2,4,6-trinitrotoluene appears to involve reduction of more than one nitro group. The lack of accumulation of monohydroxylamine and monoamine transformation products indicates that these compounds are only intermediates in the transformation of alpha TNT.

4. Aqueous munitions compounds occurring at the time of study did not affect the oxygen utilization of an unacclimated microbiological seed. Concentrations of 2,4,6-trinitrotoluene quantified in aqueous samples at the time of this study ranged from <0.2 µg/l to 1020 µg/l.

5. Benthic microbiological activity was not inhibited by TNT concentrations occurring in the sediments at the time of study. Concentrations of 2,4,6-trinitrotoluene quantified in sediment samples at the time of this study ranged from <1 mg/kg to 44,200 mg/kg.

6. The major transformation products quantified in the laboratory studies were tentatively identified as the 2,amino- 4,6 dinitrotoluene and the 4,amino-2,6 dinitrotoluene. The previously reported "hydroxylamine" transformation product was not quantitatively observed in these studies.

7. Trinitrotoluene transformation products isolated and quantified from sediment samples at IAAP and JAAP were 4-hydroxylamino-2, 6-dinitrotoluene and 2-hydroxylamino-4,6-dintrotoluene.

8. The transformation/degradation of TNT by microbial populations appears to be a co-metabolic process which requies an added carbon source.

9. The rate of transformation of TNT by microorganisms is a fairly rapid process as ≈80-90 percent of the TNT was not present in the culture extracts following three days incubation.

10. Anaerobic methane production was inhibited by TNT, but the concentrations of TNT eliciting a toxic response increased as the acclimation period increased. Concentrations of 2,4,6-trinitrotoluene quantified in the laboratory samples from this study ranged from 75 mg/l to 283 mg/l.

11. Laboratory and field studies suggested the possibilities of a greater resistance to munition compounds by Pseudomonas-like species. These organisms were predominate in the culture enrichment studies.

SECTION II

RECOMMENDATIONS

1. Further studies are needed to establish the environmental fate of TNT. This is particularly important since the transformation to aromatic polyamines is suggested.

2. The fate, toxicity and mutagenicity of diamine transformation products of alpha TNT should be delineated.

3. The toxic mechanism of TNT to microorganisms should be identified.

4. Further in situ monitoring of microbial activity should be undertaken.

5. The identification and classification of dominant indigenous, microorganisms in areas receiving munitions waste discharges should be undertaken.

6. Further studies are suggested involving enrichment for organism(s) exhibiting the ability to utilize TNT as a carbon and/or energy source.

7. An attempt should be made to establish the conditions necessary for complete oxidation of TNT by microorganisms.

8. Further aerobic and anaerobic studies should be conducted to correlate microbial activity and TNT transformation rates with high concentrations (> 100 ppm) of the munition waste.

9. Further studies are necessary to delineate the observed increase in cell density resulting when indigenous populations were grown in presence of 100 ppm versus 10 ppm TNT concentrations.

SECTION III

INTRODUCTION

An initial investigation of the potential for microbial transformation of TNT by naturally occuring sediment bacterial communities was performed as a part of the previous study program.[1] Laboratory studies of bacterial metabolism of TNT had been previously reported.[2,3,4]

Utilizing indigenous sediment populations obtained at the Iowa Army Ammunition Plant (IAAP), our previous study[1] determined that the addition of 10 mg/l TNT plus nutrients did not affect bacterial growth, however TNT was not readily utilized as a sole carbon source. Addition of other carbon sources (glucose, peptone, and fatty acids) allowed for the transformation of TNT by the aerobic heterotrophic population, with increasing nutrient concentrations resulting in lower residual concentrations of TNT and subsequent higher concentrations of the transformation products. The transformation product quantitatively identified was monohydroxyl-amino-dinitrotoluene.

Chemical analysis of sediment samples obtained at the IAAP verified the existence of this transformation product in the receiving waters. This fact, coupled with the reportedly high toxicity of hydroxylamine compared to the parent TNT[5], resulted in a more intensive study of microbiological degradation being performed as a part of the present field studies. The results obtained are reported below.

SECTION IV

FIELD SURVEY

INTRODUCTION

Surveys to investigate the effect of munitions compounds on the microbiological community were conducted at the Iowa Army Ammunition Plant (IAAP) and the Joliet Army Ammunition Plant (JAAP) during 1975. Two field surveys were made to the IAAP - one during June and the other during October. A single visit to the JAAP was conducted during the first week of June. Aqueous and sediment samples were collected during all of these surveys in order to investigate the interrelationships between alpha TNT, its related transformation products, and segments of the microbial community. Samples gathered for this purpose at the IAAP were also used as part of a comprehensive aquatic field study being performed at that plant. In the case of both IAAP and JAAP, sampling stations for this microbiological study were selected to provide varying environmental exposure to TNT bearing wastes, as well as the necessary non-exposed study controls.

Station Descriptions - IAAP (Figures 1 and 2)

Five stations in upper Brush Creek were chosen for study at the IAAP since this stream receives most of the munitions process water currently being discharged at this installation.

Station B1 is located at the head of Brush Creek, approximately 1000 meters due west of the Group 1 northern entrance. Flow at this station is intermittent, depending primarily on surface water runoff. During the June survey, velocity was 0.13 meters per second with a flow estimated at approximately 0.015 cubic meters per second. No flow was present during the October sampling period, though standing pools were observed.

FIGURE 1. SCHEMATIC OF IOWA ARMY AMMUNITION PLANT, BURLINGTON, IOWA
STREAM STATIONS 1975

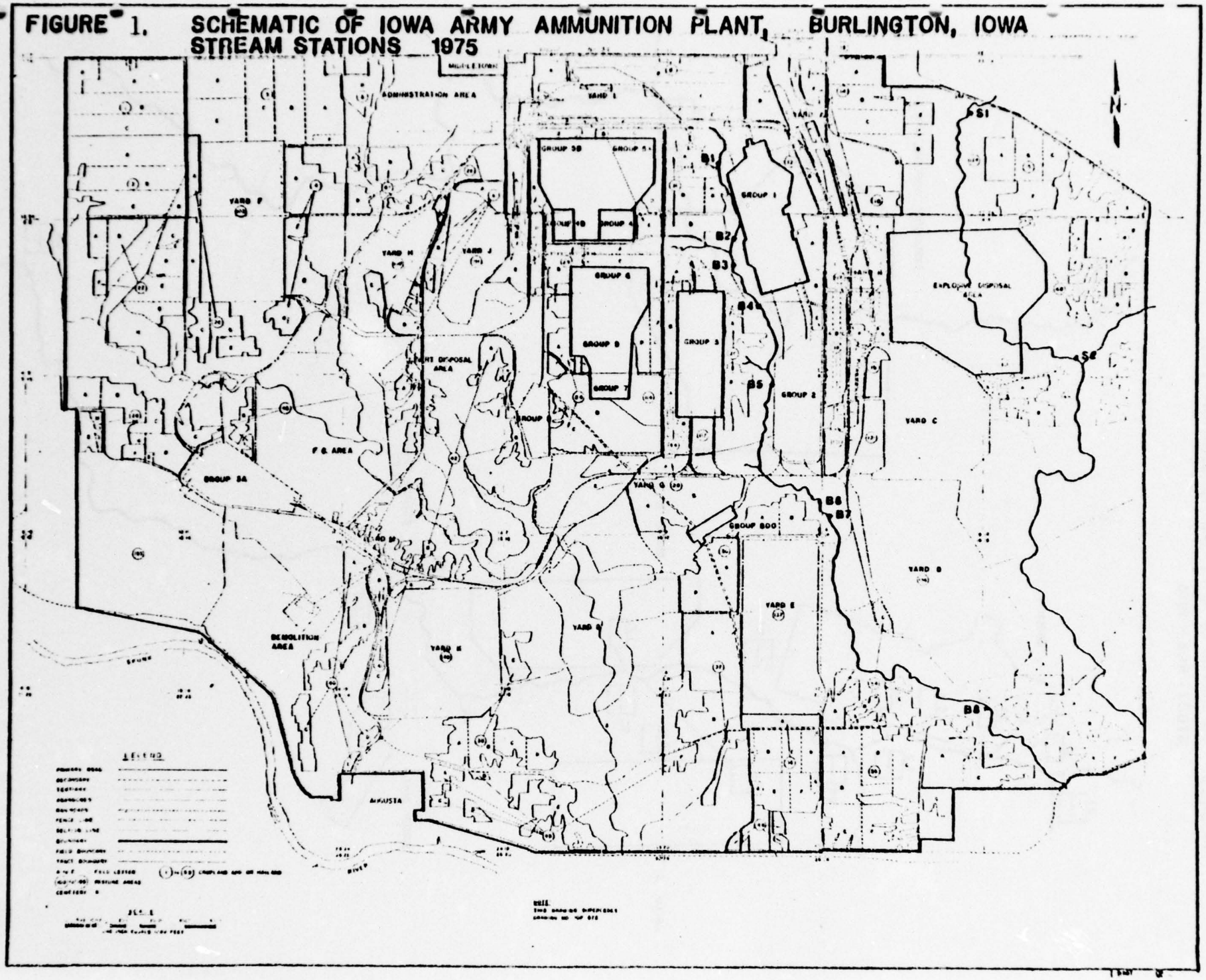
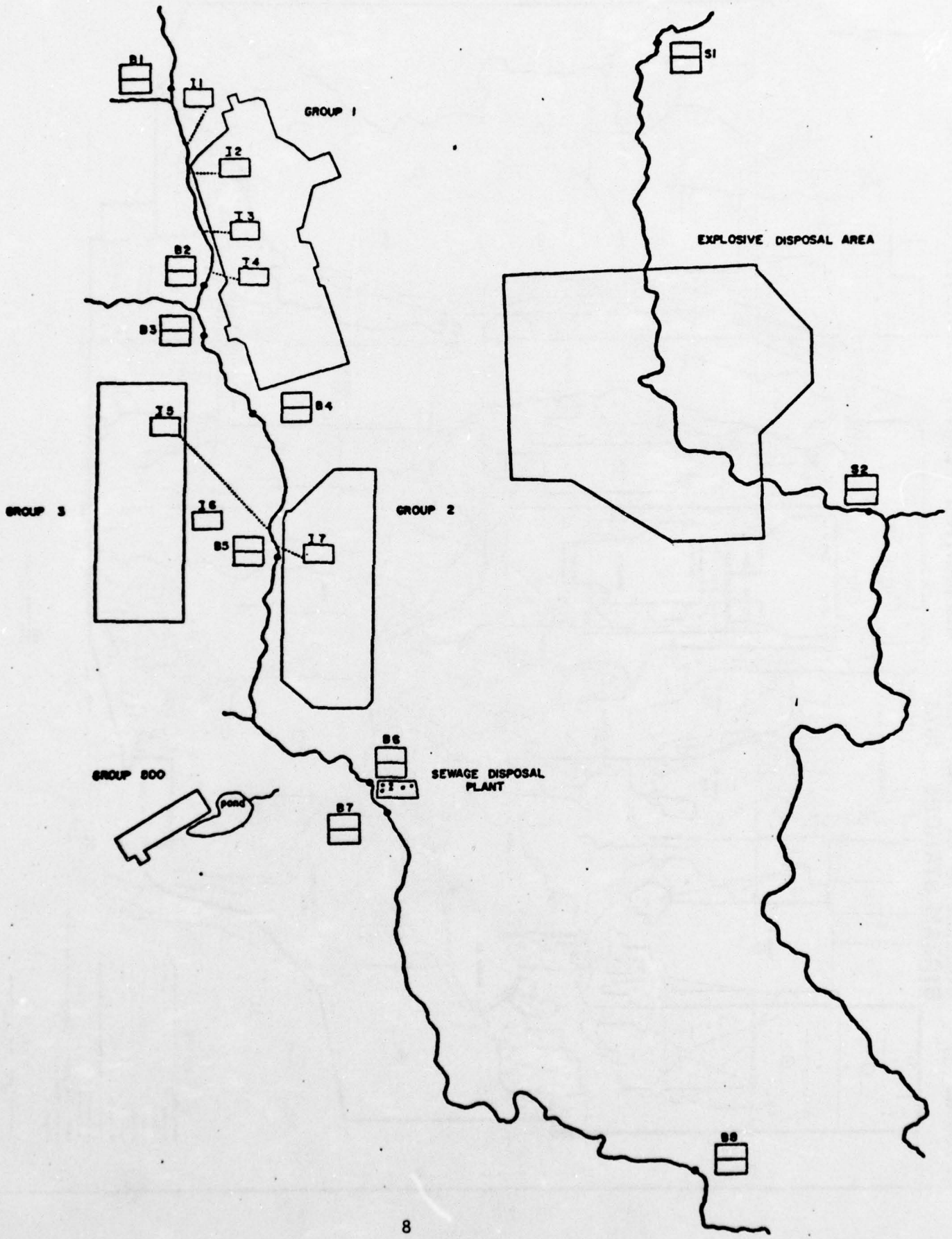


FIGURE 2. SCHEMATIC OF IOWA ARMY AMMUNITION PLANT STUDY AREA 1975



The width of the stream at this point is about 1 meter, with depth varying between 2 and 20 centimeters.

Station B2 is situated approximately 3200 meters downstream from B1, and 1400 meters west-southwest of building 1-10. It receives the effluent from four industrial outfalls, including the boiler blowdown water from the Group 1 power plant which is one of the two largest outfalls on Brush Creek. Two known sources of munitions wastes discharge upstream of B2. Width at this station is just under 2 meters, with depth varying between 5 and 20 centimeters. Velocity during the June survey was 0.13 meters per second, with a flow of 0.030 cubic meters per second. The velocity measured during October was 0.16 meters per second with an estimated flow of 0.024 cubic meters per second. The flow in Brush Creek from this station downstream is not intermittent, due primarily to the industrial process waters discharged from Groups 1, 2 and 3 as well as the treated wastewater discharged from the sewage disposal plant.

Station B3 is located approximately 900 meters south of B2. Process waters from Groups 4 and 5 flow into Brush Creek just above station B3. The width at this point is aboout 2.5 meters, with depths varying between 5 and 20 centimeters. The velocity recorded in June was 0.20 meters per second,yielding a flow of 0.059 cubic meters per second. In October these numbers were 0.08 and 0.031, respectively.

Station B4 is situated 1600 meters downstream from B3, just south of the bridge on road D. No known discharges of process water occur between stations B3 and B4, however an historic source of munitions-related compounds was discovered during the June survey just 400 meters upstream of B4. Width at this station is approximately 2 meters, with depths ranging from 8 to 20 centimeters. Velocity during the summer survey was 0.11 meters per second, with a corresponding flow of 0.031 cubic meters per second. During the fall the recorded velocity was 0.16 meters per second, and the flow was estimated at 0.045 cubic meters per second.

Station B5 was the furthest downstream for the microbiological studies. It is located approximately 2400 meters downstream of station B4, just below the munitions wastewater outfall of IAAP Group 2. Width at this station is approximately 2 meters, with depths varying between 2 and 10 centimeters. The velocity recorded in June was 0.21 meters per second, with a flow of 0.027 cubic meters per second. In October the velocity was somewhat higher, at 0.63 meters per second, with a corresponding flow of 0.087 cubic meters per second.

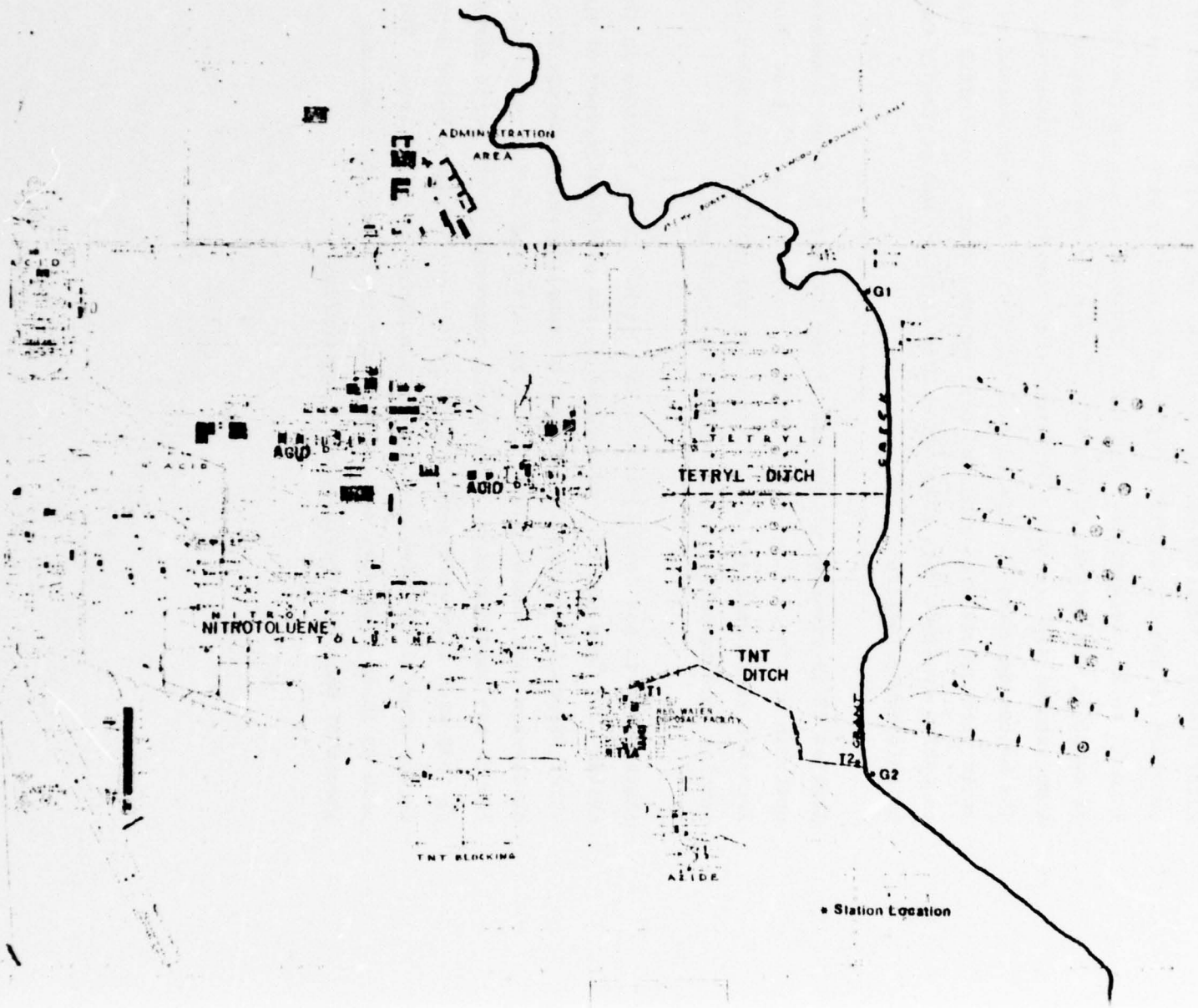
Station Descriptions - JAAP (Figure 3)

Sampling stations selected at the JAAP were located on Grant Creek and the open wastewater channel known as "TNT Ditch". The concentrations of munitions compounds discharged into the TNT Ditch and lower Grant Creek are much higher than those measured at the IAAP, so the opportunity to study much higher environmental stress loadings was present at the JAAP.

Station G1 is situated on Grant Creek, upstream from the tetryl area, 155 meters due west of the intersection of Base Line and Blodgett roads. This station served as the control for the remaining stations at JAAP. (Width of the stream at this station is approximately 4 meters, while the depth at the point where sediment core samples were taken was approximately one half meter.) Stream velocities and flows were not determined at G1 or any other station at the Joliet AAP.

Station G2 is situated on Brant Creek 160 meters due west of the intersection of West TNT Road and Blodgett Road. The point of sampling at this station is above 10 meters downstream of the confluence of TNT Ditch and Grant Creek, in a zone where mixing is reasonably complete. Width of Grant Creek at this station is 6 to 7 meters. The water and sediment samples were collected where stream depth was approximately one half meter.

FIGURE 3. SCHEMATIC OF JOLIET ARMY AMMUNITION PLANT, JOLIET, ILLINOIS



Station T1 is located 10 meters downstream of the outfall of the Red Water Disposal Facility on the TNT Ditch. Width of the stream at this location is about 2 meters. Depth where samples were collected varied from 10 to 15 centimeters. The water temperature during the two days of sampling was between 30 and 40°C. This was the only station where temperatures significantly different than ambient were observed. The bottom deposits in this section of the TNT Ditch consisted of large rocks with accumulated sand and silt between them. Collecting representative sediment core samples at this location was seriously hampered by this course stratum.

Station T2 is situated at the end of the TNT Ditch, about 2 meters upstream of its confluence with Grant Creek. Stream width at this location is approximately 3 meters. Average depth at the point where samples were taken was approximately one half meter.

Station T1A is not located on the TNT Ditch. It is situated in the center of a "red water" pond located at the northwest corner of the Red Water Disposal Facility. The point of sampling was 80 meters due west of storage tank number 860-3. Flow to this pond is intermittent, though the concentration of munitions compounds in the waste during periods when flow does occur is apparently quite high. Water depth in the pond at the time of sampling was about 20 centimeters. The pond is in an open area and consequently is subject to a maximum amount of photochemically stimulating sunlight.

FIELD METHODOLOGY

Chemistry

Iowa AAP -

Each of the stream and industrial stations were sampled five times during both the summer and fall survey periods. Sampling was conducted only during periods of production line operation. A summary of the production activities of each IAAP group is outlined in Table 1. It is noteworthy that according to IAAP personnel, plant operations were at approximately 10 percent of rated capacity. Thus, the impact of the processing installations on stream conditions are approaching the lowest that could exist without a full scale shutdown of the munitions processing parts of the plant.

Water samples were collected daily during the operation of the processing facilities but without regard for exact time of day. At each stream and industrial station, approximately four gallons of water was collected and composited in plastic buckets. The sample was then poured off into five subsample containers, one for each preservative to be used and a fifth for analyses to be performed immediately upon returning to the field laboratory. Temperature was recorded at the sampling site, although no deviation from ambient (i.e. approximately 25°C during the summer survey and 15°C during the fall survey) was observed at any station except industrial station 1. After pouring off the sample into the appropriate subsample vessels, all containers were stored in ice chests until received at the field laboratory.

IAAP contractor personnel kindly provided laboratory facilities for the summer and fall survey periods, so the analysis of samples for certain parameters was conducted immediately upon receipt of the samples from the field. These included pH, alkalinity, dissolved oxygen, BOD and specific conductance. A one liter polyethylene container which had been filled to exclude air space was used for these analyses. A second one

Table 1. INDUSTRIAL OPERATIONS AT IOWA ARMY AMMUNITION PLANT - JUNE 1975

Facility	Process Elements	Production Status
Group 1	Octol	Active
Group 2	Composition B, Octol	Active
Group 3	Composition B, Metal Processing	Active
Group 3A	Composition B	Active
Group 4	Assembly	Active
Group 5A	TNT	Active
Group 5B	(Not Available)	Inactive
Group 6	Detonators	Inactive
Group 7	Boosters, Black Powder	Inactive
Group 8	Fertilizer	Inactive
Group 9	Fuses, Black Powder	Active
Group 800	Composition B, Metal Processing	Active

liter polyethylene container filled to the brim was transported and stored at 4°C until analysis of total solids, total suspended solids, chloride, sulfate, hardness, sodium and potassium was completed in Ann Arbor. The subsample for trace metal analysis was stored in polyethylene and preserved by adding 10 ml reagent nitric acid per liter of sample. A 2.4 liter glass container was used to store the subsample for nutrient analysis. Reagent sulfuric acid was added at a concentration of 2 ml per liter of sample to inhibit biological activity and retard the transformation of nitrogen forms. This nutrient subsample was also transported and stored at 4°C until all required analyses had been completed. The subsample for munitions compounds analysis was stored in four liter brown glass bottles with teflon lined caps. Upon receipt in the field laboratory, the samples were poured off to the 3.8 liter mark and 50 ml of benzene ("Distilled in Glass", Burdick and Jackson, Muskegon, MI) was added. The sample was then stirred for ten minutes at a sufficiently rapid rate to insure uniform dispersion of the benzene solvent. This procedure serves two purposes. First, biological alteration of the munitions compounds is inhibited due to the extreme biocidal character of benzene. Secondly, the munitions compounds and their benzene soluble transformation products are isolated from the aqueous phase, which retards chemical alteration of the compounds. After ten minutes of stirring, the sample was capped and prepared for shipping back to Ann Arbor, where the extraction procedure would be completed. As a further means of stabilizing the munitions compounds, all such partially extracted samples were transported and stored at 4°C until the extraction procedure had been completed.

After the five daily water samplings had been gathered, and the preliminary water quality of the industrial effluents established, a diurnal sampling program was initiated in order to verify that the stream water quality did not vary significantly during the period when normal sampling was not performed. Automatic samplers were placed at stream stations B1 and B8. Samples were taken once a hour for 48 hours. The samples were stored on ice inside the automatic sampler

to minimize changes in water chemistry. They were collected by laboratory personnel and analyzed for pH and specific conductance every 12 hours. Since production at the IAAP was limited to the day and afternoon shifts during the two survey periods, and since the summer diurnal study confirmed that the water quality of Brush Creek did not change significantly during the period of no sampling activity, a diurnal study was not performed during the fall survey period.

Sediment samples were collected after all water sampling was complete. At the outset of the summer survey period a zone was marked off from which sediment samples were to be gathered. Care was taken to insure that these zones were not disturbed during other stream sampling. The same zones were used to gather sediments for both the summer and fall surveys. As a result of this, differences in sediment chemistry at a given station between summer and fall samplings may be interpreted as a change in the character of the stream bottom deposits.

Two inch diameter polycarbonate core tubes were used to gather all sediment samples. Each tube has air-tight end caps which are removed before pressing the transparent sleeve into the sediment. Once the tube has been pressed to the desired depth, the top is capped and the tube is withdrawn with core sample intact. The bottom is then capped and the sample is frozen. Three core samples were gathered from each stream station during each of the two survey periods. The cores were frozen with dry ice immediately after sampling and stored in this condition until analysis could begin.

Joliet AAP -

Water and sediment samples taken at the Joliet AAP were collected and processed just as described for the IAAP. Field collections were made during hours of plant operation, and industrial discharges from the

acid processing area as well as the nitrotoluene processing area were observed during both days of sampling. As previously mentioned, only one survey was conducted at the JAAP. Samples for all aqueous phase analyses were collected on 2 June 1975, while sediment cores were taken on 3 June 1975.

At both JAAP and IAAP, sediment redox conditions were measured in selected core samples to further define the effect of munitions compounds on this phase of the environment. This was accomplished by inserting pH and redox electrodes into freshly taken sediment cores at predetermined depths. Specially modified core tubes were used for these selective samples, and all redox measurements were performed prior to freezing the samples for preservation purposes.

Microbiology

Iowa and Joliet AAP -

Aqueous samples for microbiological analyses were collected in 125 ml sterile glass bottles and analyzed within six to eight hours. Sediment microcores were taken with sterile 50 cc plastic disposable syringes following removal of the luer-lock tip. The top half-centimeter of the sediment core was removed and suspended in 200 ml sterile, distilled, buffered water. Samples were maintained at approximately 4°C and analyzed within 30 days.

SECTION V

CHEMISTRY

ANALYTICAL PROCEDURES

Aqueous Phase

In order to evaluate the impact of the AAP on the receiving waters, an extensive characterization of the water quality of the receiving stream, as well as a characterization of the industrial effluents, was undertaken. The parameters monitored included the following:

Dissolved Oxygen	Suspended Solids
pH	Total Solids
Alkalinity	Chloride
Specific Conductance	Sulfate
Biological Oxygen Demand	Sodium
Chemical Oxygen Demand	Potassium
Total Organic Carbon	Hardness
Cadmium	Nitrate-N
Chromium	Nitrite-N
Iron	Ammonia-N
Lead	Kjeldahl-N
Manganese	Total Phosphorus
Mercury	Munitions Compounds

General Water Quality Parameters -

Sampling of the aqueous phase for these parameters has been described in a previous section of this report. However, some additional comments are noteworthy here. All sample containers had been acid-washed and rinsed with copious amounts of distilled water. As noted earlier, samples for metal analysis were preserved with reagent grade

nitric acid. Samples for nutrient, COD, and TOC analysis were preserved with reagent sulfuric acid and refrigerated. All other samples were preserved by refrigeration at 4°C from the time of sampling to the completion of analysis in the laboratory.

In general, all methods of chemical analysis employed in the characterization of aqueous samples were taken from the three most widely accepted compilations of such procedures[6-8]. Where methods were unavailable or insufficient to provide the desired information, particularly with respect to munitions compounds, alternate analytical procedures were employed after their accuracy and precision had been statistically verified. A brief synopsis of the analytical methodology is contained in the following paragraphs.

Measurements of dissolved oxygen were made, in the in situ stream determinations and in the analysis of biochemical oxygen demand, with a polarographic-type gas sensing probe which utilizes a semipermeable fluorocarbon membrane. Hydrogen ion concentration was measured with a glass membrane/calomel combination electrode and digital pH meter capable of 0.01 unit resolution. This appartus was also used in the standard acid tritration for alkalinity. Chloride ion concentration was determined by a method adapted from the flouride ion selective electrode method listed in the EPA manual[6]. A chloride ion selective electrode from Corning Scientific Instruments (model 476126) was used in conjunction with a silver/silver chloride reference electrode. The reference cell was fitted with a secondary salt-bridge containing 1.0 M potassium nitrate to prevent chloride bleed into the sample solution. Calibration of the device was accomplished by standard addition in order to compensate for matrix and temperature effects. Sulfate ion concentration was determined by the barium sulfate suspension technique outlined in the EPA reference[6]. Suspended solids were measured using Millipore AP40 glass fiber mats, pressure filtration and drying to constant weight at 105°C. Total solids were measured by evaporationg a 100 ml aliquot of sample to dryness at 105°C.

Biological oxygen demand in the AAP water samples were measured according to the serial dilution procedure specified in APHA Standard Methods[7]. The samples were set on the same day as collected, and incubated for

five days. Chemical oxygen demand was determined by the dichromate/sulfuric acid digestion method. The oxidant was 0.025 N dichromate, providing an effective detection limit of approximately 5 mg/l. Consumption of the oxidant was measured spectrophotometrically. Total organic carbon was determined using an Oceanography International total carbon system. With this system, an aliquot of acidified sample is sealed in a 10 ml ampule containing persulfate and digested overnight in a pressure vessel at 175°C. The persulfate oxidizes the organic carbon to CO_2. The ampules are broken and the CO_2 flushed through an infrared detector interfaced with a digital integrator.

Nitrogen was measured in four forms in the aqueous phase. Nitrate-nitrogen was reacted with brucine sulfate in acidic media to produce a colored complex which was measured spectrophotometrically. Nitrite-nitrogen was similary determined, though in this case the colored complex results from the diazo coupling of sulfanilic acid and naphthylamine hydrochloride in the presence of nitrite and excess hydrogen ions. Reduced nitrogen forms were determined by the Kjeldahl method. This method employs a mercury catalyzed sulfuric acid digestion followed by distillation into boric acid and a potentiometric endpoint titration. Ammonia concentrations were measured with a potentiometric-type gas sensing probe. Determination of ammonia with this device is now an accepted EPA procedure[6]. Evaluation of the ammonia probe by the U.S. Environmental Protection Agency and by exhaustive tests in our own laboratory reveals it to be equal in accuracy and precision to the indophenol blue method commonly employed for low levels of ammonia.

Total phosphorus levels in the aqueous phase were determined on the whole-water samples after a persulfate/sulfuric acid digestion. The digestate was subjected to analysis using either the ascorbic acid or vanadomolybdophosphoric acid technique outlined in the APHA water analysis manual, depending on the phosphorus level.

Metal analysis of the aqueous phase was accomplished by atomic absorption spectrophotometry. Total concentrations of the metals cadmium, chromium, iron, lead, manganese, and mercury were determined in the acidified water samples. Calcium and magnesium where determined on filtered water samples. High temperature flameless AAS was employed for all metals except calcium,

iron, magnesium, and mercury[9]. Mercury was analyzed using the cold vapor atomic absorption technique developed by Hatch and Ott[10]. Calcium, iron and magnesium were analyzed using conventional air-acetylene flame AAS[6-7]. Calcium and magnesium values so determined were used to calculate hardness[7]. The method of standard addition was utilized whenever necessary to compensate for matrix effects on instrument calibration. Sodium and potassium were determined by flame emission spectrophotometry.

Munitions Compounds -

Analysis of AAP water samples for munitions compounds followed closely the analytical methodology of the 1974 study [1]. The benzene layer in the water samples brought back from the field was removed and each sample was re-extracted with two additional 50 ml aliquots of benzene. The combined extract from each sample was dried with anhydrous sodium sulfate and concentrated to approximately 5 ml by passing nitrogen over the liquid surface while heating the extract on a water bath. The 5 ml concentrate was administered to the top of a 1 cm by 7 cm high column of fully activated silica gel (Davison grade 923). The column was wet packed in benzene. One hundred milliliters of 20 percent (v/v) ethyl ether in benzene was used to elute the components of interest. Studies conducted last year indicated that under these conditions, compounds with base character less than or equal to aminodinitrotoluene would be eluted in this cleanup. This includes such compounds as the mononitrotoluenes, dinitrotoluenes, trinitrotoluenes, mono-, di - and trinitrobenzenes, tetranitroazoxytoluenes, hydroxylaminodinitrotoluenes and the two monoamino reduction products of TNT. It should be noted here that the diamino transformation products, as well as other polyamines, would not be recovered in this cleanup procedure.

The elutriate is collected and concentrated using the previously described procedure to less than 5 ml. At this point the concentrate is transferred to a 5 ml vial and the extract is taken to dryness. The sample extract is stored in this condition at -20°C until analysis by vapor phase chromatography (VPC). At such time, the extract is taken up in a predetermined

volume of benzene and an aliquot of this concentrate is administered to the gas chromatograph.

The VPC system used for this study is somewhat different than that used in 1974[1]. Several researchers have reported that 1, 3, 5 trinitrobenzene is an important photolysis product of 2, 4, 6- TNT[11-12]. As a consequence, it was deemd important to be able to differentiate 2, 4, 6 - TNT from 1, 3, 5- TNB in the environment. In investigating the applicability of the existing extraction/cleanup method for TNB analysis, it was learned that a 5 percent Dexsil 300 liquid phase could not chromatographically resolve, 2, 4, 6 - TNT from 1, 3, 5, - TNB, despite numerous manipulations of chromatographic conditions. A 10 percent liquid loading of G.C. grade SE-30 was found to resolve these two compounds and still provide adequate chromatographic chracteristics for the other munitions-related compounds of interest. The chromatographic system employed for these analyses is detailed in Figure 4. The resolutuion capability of this VPC system is shown in Figure 5. Chromatograms of representative water and sediment extracts appear in Figures 6 and 7, respectively.

A recovery study performed on the extraction/cleanup/VPC analysis system described above confirmed that the method is essentially quantitative for 2, 6 - dinitrotoluene, 2, 4 - dinitrotoluene, 1, 3, 5 - trinitrobenzene, 2, 4, 6 - trinitrotoluene and 4 - hydroxylamino - 2, 6 - dinitrotoluene, with recovery efficiencies ranging from 95 to 100 percent.

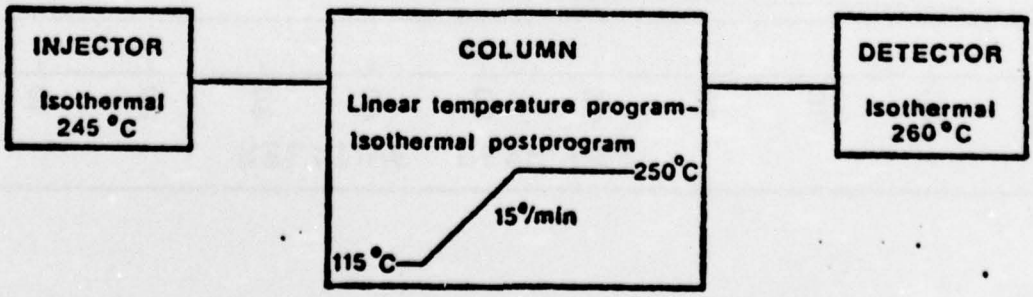
At the JAAP, where large volumes of "red water" are subjected to sunlight. another photolysis product was considered worthy of study. Sitzimann and Kaplan have reported that 2, 4, 6 - trinitrobenzaldehyde is produced in reasonable yields during the photolysis of aqueous solutions of 2, 4, 6 - trinitrotoluene[12]. However, this compound is quite reactive and probably does not exist long in sedimentary environments. Consequently, only water samples from Joliet AAP were analyzed for this compound.

In attempting the analysis of this transformation product, it was found that it could not be successfully chromatographed in the vapor phase on

FIGURE 4. ANALYTICAL SYSTEM FOR VAPOR PHASE CHROMATOGRAPHY USING SE-30 COLUMN

INSTRUMENT	VARIAN 1860
INJECTION	ON COLUMN
SAMPLE SIZE	4 µl
COLUMN	
LENGTH	180 cm
O.D.	3 mm
COATING	G.C. SE-30
LOADING	10% (w/w)
SUPPORT	HP CHROMOSORB W AW-DMCS
MESH	80/100
CARRIER GAS	NITROGEN (LINDE UHP)
FLOW	40 ml per minute
DETECTOR	HYDROGEN FLAME IONIZATION

TEMPERATURE CONDITIONS



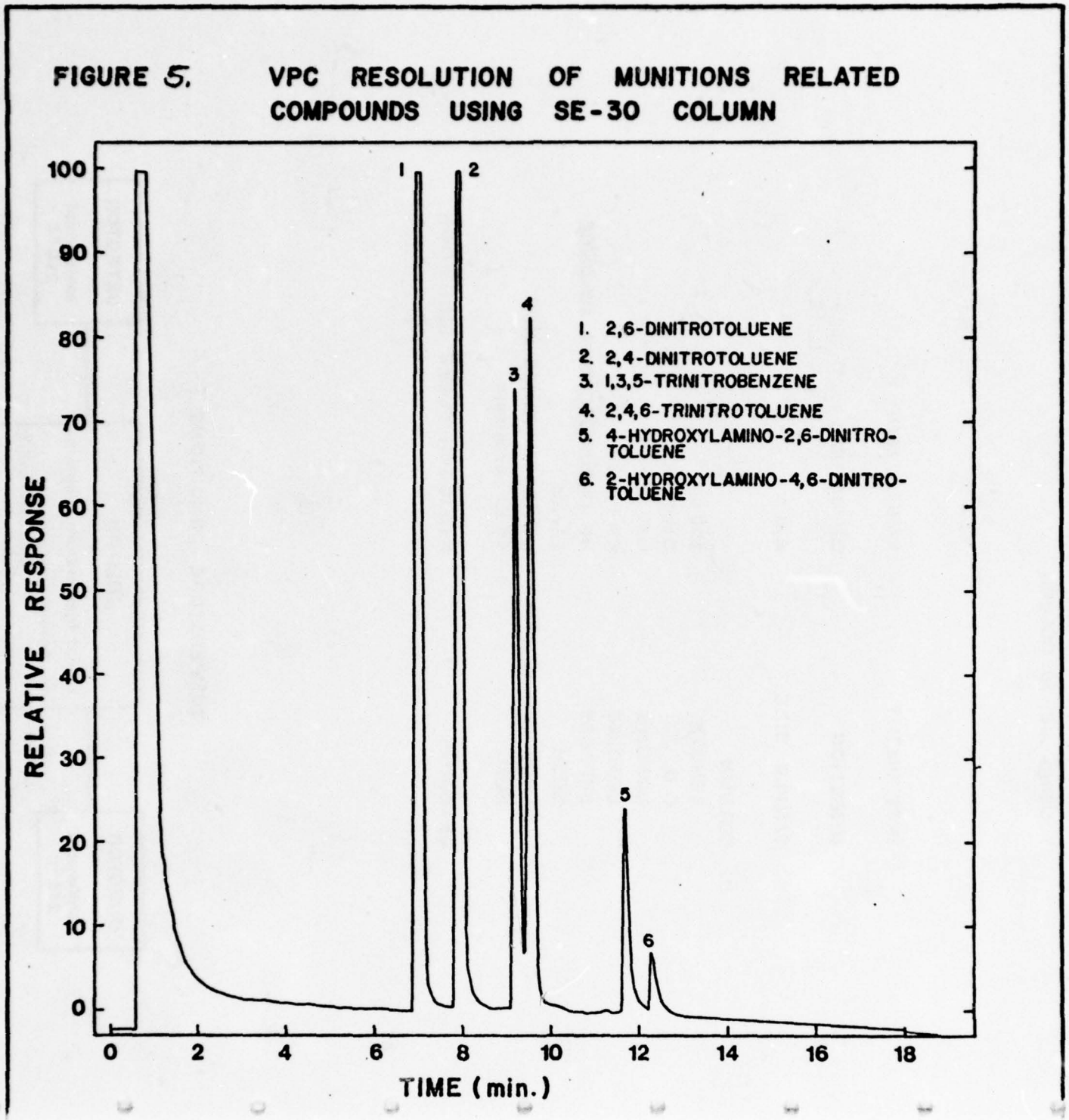


FIGURE 5. VPC RESOLUTION OF MUNITIONS RELATED COMPOUNDS USING SE-30 COLUMN
RELATIVE RESPONSE
TIME (min.)
1. 2,6-DINITROTOLUENE
2. 2,4-DINITROTOLUENE
3. 1,3,5-TRINITROBENZENE
4. 2,4,6-TRINITROTOLUENE
5. 4-HYDROXYLAMINO-2,6-DINITRO-TOLUENE
6. 2-HYDROXYLAMINO-4,6-DINITRO-TOLUENE

FIGURE 6. VPC RESOLUTION OF COMPONENTS IN TYPICAL WATER EXTRACT

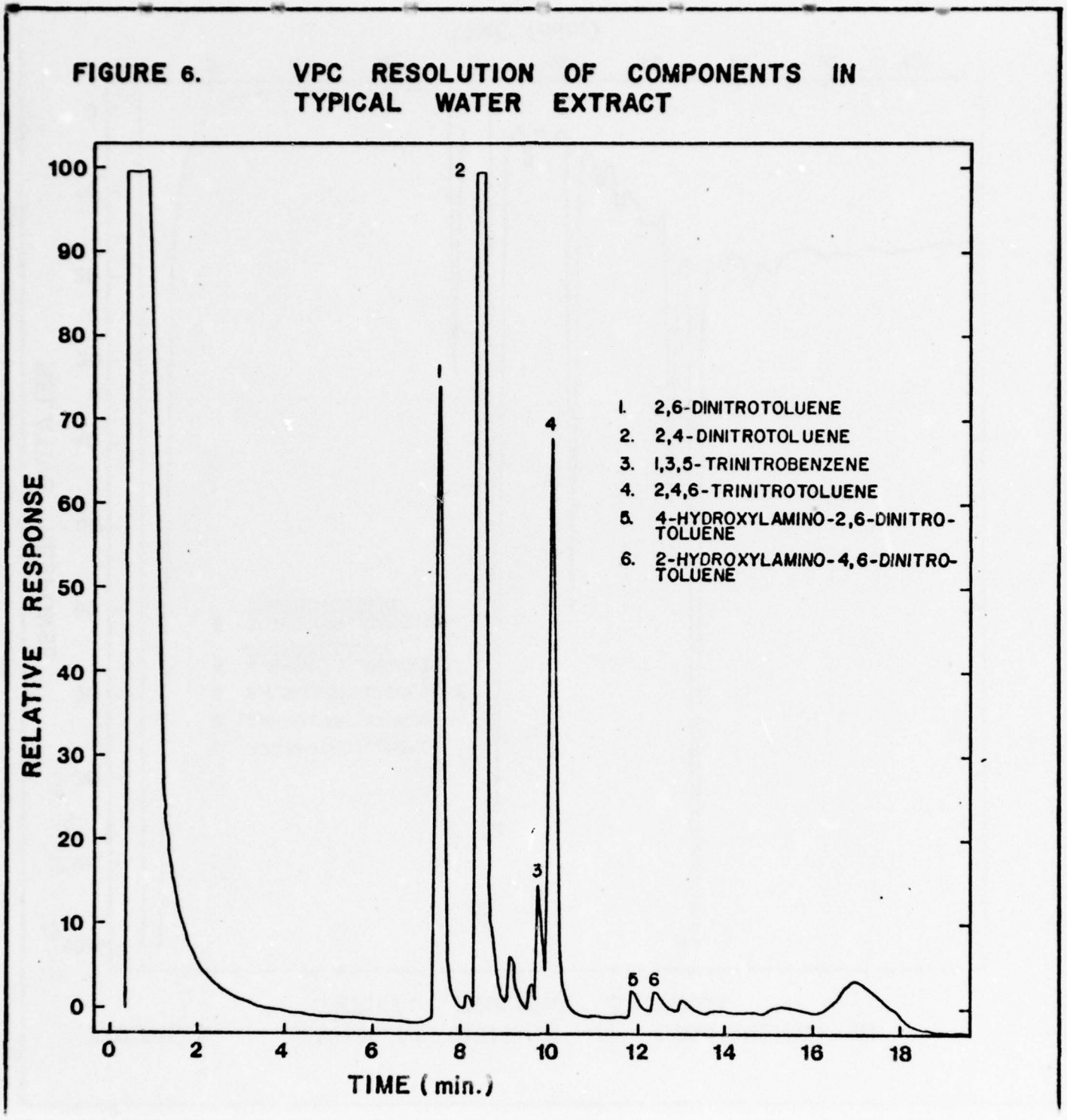
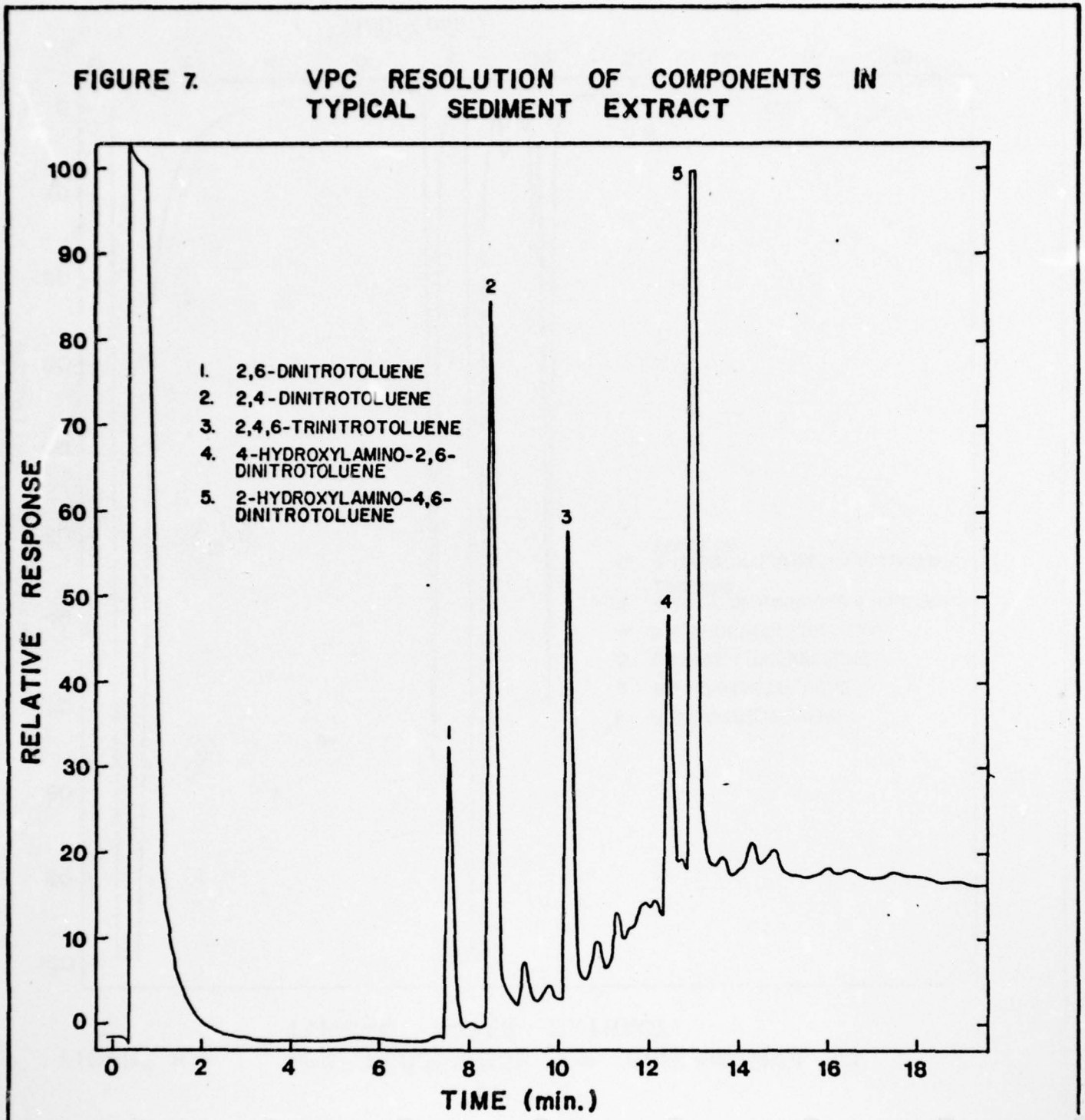


FIGURE 7. VPC RESOLUTION OF COMPONENTS IN TYPICAL SEDIMENT EXTRACT



a 10 percent SE-30 column. However, a liquid loading of 5 percent Dexsil on HPCW was found to be satisfactory for the quantitative analysis of this compound and consequently extracts from aqueous samples taken at JAAP were each analyzed by vapor phase chromatography on both columns. A description of conditions used with the Dexsil column appears in Figure 8 . The resolution capabilities of this system are represented in Figure 9 .

It should be noted here that although cyclotrimethylene trinitroamine (RDX) and, to a lesser extent, cyclotetramethylene tetranitramine (HMX) are processed at the LAAP as co-explosives with alpha TNT in mixtures such as Composition B and Octol, only 5 mg of each compound was available from the Army for use in analytical method development and as quantitative standards for sample analysis. Such quantities were insufficient even for adequate method development and verification so RDX and HMX were deleted from the list of organic compounds under study during the current project.

Sediment Phase

Sampling of the sediment phase at the AAP has been described in an earlier section of this report. The samples were thoroughly frozen when received from the field and were stored in this condition until processing commenced. Freezing sediment material often disrupts the mineral morphology, but it was felt that the analyses to be performed on these samples would not be adversely affected and the low temperature preservation would minimize the decomposition of any munitions-related compounds present. The following parameters were determined on the sediment samples:

Total Solids	Cadmium
Total Volatile Solids	Chromium
Chemical Oxygen Demand	Iron
Hexane Extractables	Lead
Kjeldahl Nitrogen	Manganese
Nitrate+Nitrite Nitrogen	
Mercury	Total Phosphorus
Munitions Compounds	

FIGURE 8. ANALYTICAL SYSTEM FOR VAPOR PHASE CHROMATOGRAPHY USING DEXSIL 300 COLUMN

INSTRUMENT	VARIAN 1860
INJECTION	ON COLUMN
SAMPLE SIZE	4 µl
COLUMN	
LENGTH	180 cm
O.D.	3 mm
COATING	DEXSIL 300
LOADING	5% (w/w)
SUPPORT	HP CHROMOSORB W AW-DMCS
MESH	80/100
CARRIER GAS	NITROGEN (LINDE UHP)
FLOW	50 ml per minute
DETECTOR	HYDROGEN FLAME IONIZATION

TEMPERATURE CONDITIONS

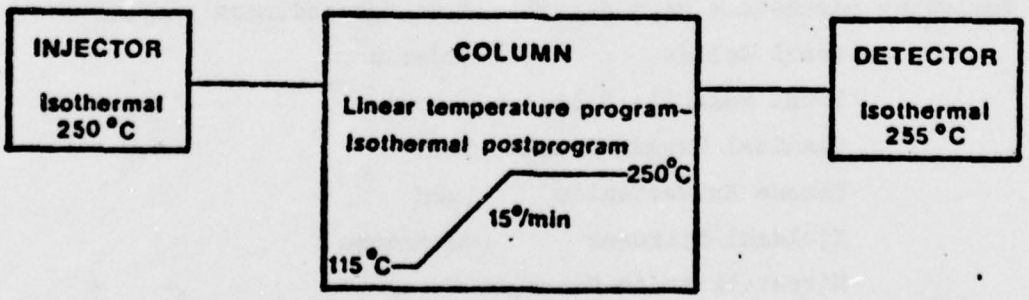
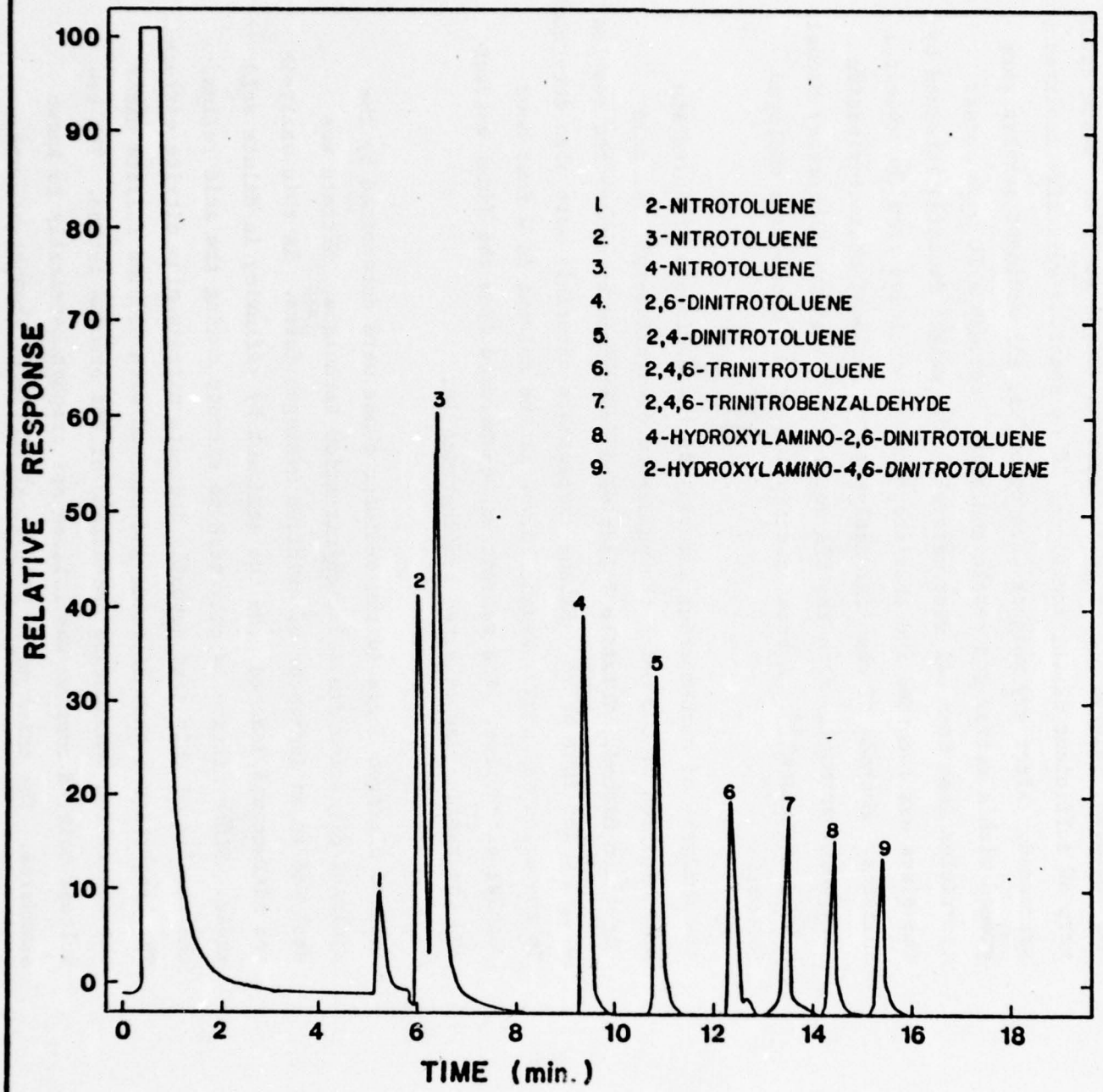


FIGURE 9. VPC RESOLUTION OF MUNITIONS RELATED COMPOUNDS USING DEXSIL 300 COLUMN



General Sediment Parameters -

The sediment samples were thawed for processing and extruded from the polycarbonate core liners. Physical descriptions were made immediately and the core samples were sectioned according to depth from the water/sediment interface. For the present study, the upper 10 cm section was isolated, weighed and dried to constant weight at 50°C. Where cores were of sufficient depth, additional 10 cm sections were also isolated and processed. After dry weights were recorded, all sediment samples were ground with a mortar and pestle and sieved through a 20 mesh screen (particles less than 841 micrometers). The weight fraction retained by the sieve was recorded and this fraction was excluded from the chemical analyses. Methods of chemical analysis for sediment characterization were taken primarily from the EPA reference "Chemistry Laboratory Manual: Bottom Sediments"[13]. A brief description of the procedures employed follows.

The analysis of carbonaceous material in the sediments included the determination of COD using the potassium dichromate-sulfuric acid digestion method. Volatile solids were determined by ashing the samples at 575°C for four hours. Hexane extractable materials were also determined in the dried sediment. Hexane served as the solvent in a four hour soxhlet extraction. The solvent was evaporated from the final extract and the residue was measured gravimetrically.

Reduced nitrogen forms in the sediment phase were determined by the Kjeldahl digestion/distillation/titration technique. Nitrate was analyzed as an indicator of oxidized nitrogen forms. In this analysis the nitrate was leached from the sediment by refluxing in dilute acid media. Since nitrite is converted to nitrate during the acid reflux, the results of this test actually indicate nitrate plus nitrite nitrogen. The leachate was then filtered and reacted with brucine sulfate under the controlled temperature conditions of the brucine method. The resultant colored complex was related spectrophotometrically to known standards. The third major biological nutrient, phosphorus, was measured in the sediments with the vanadomolydophosphoric acid test after the samples had undergone a persulfate/sulfuric acid digestion[7,13].

Sediment samples for metal analysis, with the exception of mercury, were prepared by dry ashing at 575°C for four hours, acid leaching the residue with a nitric acid/hydrogen peroxide solution, and removing the undissolved residue by filtration. The filtrate was analyzed for cadmium, chromium, iron, lead, and manganese using conventional air-acetylene flame atomic absorption spectrophotometry [13,14]. Mercury analysis was performed on samples prepared by wet digestion [15]. The finely divided samples were allowed to react overnight with fuming nitric acid and potassium dichromate. After digestion was complete, the excess dichromate was reduced with hydroxylamine hydrochloride. Reduction of the mercury with stannous chloride was followed by detection of the resulting elemental mercury using the cold vapor atomic absorption method[10].

Redox conditions in selected IAAP and JAAP core samples were measured with a platinum wire electrode and a micro glass membrane/calomel reference combination pH electrode. Both electrodes were pressed into the cores at predetermined depths and allow to equilibrate for ten minutes or until no further drift was observed, whichever came first. A 0.0033 M solution of potassium ferrocyanide and potassium ferricyanide was used to calibrate the redox cell. This is essentially the system used by Zobell[16].

Munitions Compounds -

Sediment samples were air dried at 50°C specifically to retard thermal degradation of munitions-related compounds. Twenty grams of the ground and sieved sediment samples were extracted with benzene in a Soxhlet extractor for four hours, after which the extract was concentrated and cleaned up according to the method outlined in the aqueous phase section. Analysis of the extract by vapor phase chromatography was also essentially the same as for the water samples, though the solvent make-up volumes and the amount introduced into the gas chromatograph were adjusted to compensate for the greater amount of matrix material (primarily oils) found in the sediment extracts. Spiked samples were used to verify quantitative recovery (95-100%) of the compounds of interest.

RESULTS AND DISCUSSION - IAAP

Aqueous Phase

General Water Quality -

Mean values for general water quality parameters for each survey period are presented in Tables 2 and 3 . These concentrations represent average values of the five samples gathered during a survey period. Where levels are below the analytical detection limit in all five daily samples, the mean value appears as a "less than" number in the tables. Where one or more of the daily samples had detectable concentrations, the "less than" values were averaged as if the component of interest had been observed at the detection limit (e.g. in this case, <0.001 would be averaged as 0.001). This allows differentiation of those stations where no detectable quantities were found from other stations at which analysis of one or more daily samples revealed detectable quantities of the component of interest. Thus, throughout the chemistry section of this report, where a "less than" sign is observed in the tables of mean values or tables of statistical values related to mean values such as standard deviation, the indication is clear that no concentrations of the component of interest were detected in any of the samples taken at a given station during a given survey period.

A review of Table 2 reveals that during the summer sampling the water quality of Brush Creek at stations B2 through B8 was affected by environmentally significant enrichment in two general areas: 1) major dissolved solids; and 2) biostimulating nutrients. Increases in chloride, sulfate, sodium, and to a lesser degree potassium and hardness were responsible for an average dissolved solids burden in this reach of Brush Creek which was approximately 30 percent higher than at station B1. Average concentrations of carbon, reduced and oxidized forms of nitrogen, and total phosphorus were also higher in this stretch of Brush Creek. The high concentrations of total phosphorus and nitrate-

Table 2. AQUESOUS PHASE CHEMICAL DATA
IOWA ARMY AMMUNITION PLANT JUNE 1975
BRUSH CREEK STATIONS - MEANS

Parameter	Units	B1	B2	B3	B4	B5	B6	B7	B8
Specific Conductance	µmhos/cm	520	740	720	630	550	870	670	570
Total Solids	mg/l	355	476	467	412	382	566	428	365
Total Suspended Solids	mg/l	31	13	14	8	17	4	7	3
pH	SU*	8.25	9.30	9.30	9.30	9.10	8.80	8.60	8.90
Total Alkalinity	mg/l as $CaCO_3$	184	153	153	152	131	141	133	145
Chloride	mg/l	37.1	109	97.3	72.8	58.6	162	110	68.8
Sulfate	mg/l	38	73	71	71	61	61	61	60
Total Hardness	mg/l as $CaCO_3$	280	159	164	151	149	194	181	185
Calcium	mg/l	65.9	33.7	35.0	34.1	33.9	42.0	38.8	42.9
Magnesium	mg/l	28.2	18.2	18.6	16.1	15.7	21.8	20.6	19.1
Sodium	mg/l	12	80	99	84	61	105	70	45
Potassium	mg/l	0.5	2.5	2.8	3.2	3.1	4.2	4.8	4.0
Dissolved Oxygen	mg/l	8.5	8.4	8.2	8.6	8.4	8.5	9.2	10.2
BOD	mg/l	2	2	1	2	4	2	4	2
COD(A)	mg/l	<8	14	14	23	18	10	15	10
TOC	mg/l	4	15	25	16	10	12	9	6
Kjeldahl-N	mg/l	0.8	0.7	0.7	0.8	2.0	0.6	0.9	0.5
Ammonia-N	mg/l	0.084	0.083	0.11	0.12	1.5	0.11	0.42	0.075
Nitrite-N	mg/l	0.028	0.009	0.010	0.016	0.11	0.010	0.008	0.005
Nitrate-N	mg/l	3.6	4.1	3.7	3.2	4.3	2.4	4.6	4.1
Total Phosphorus	mg/l	0.073	0.58	0.56	0.52	0.47	0.59	0.94	0.72

Table 2 (continued).

Parameter	Units	B1	B2	B3	B4	B5	B6	B7	B8
Cadmium[(B)]	mg/l	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Chromium	mg/l	0.005	0.163	0.122	0.095	0.044	0.042	0.042	0.013
Iron	mg/l	1.13	0.58	0.65	0.41	0.091	0.32	0.48	0.25
Lead	mg/l	0.003	0.003	0.003	0.002	0.003	0.002	0.003	0.002
Manganese	mg/l	0.154	0.115	0.111	0.063	0.135	0.035	0.086	0.028
Mercury	mg/l	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0007	0.0001

*Median Value

(A) "less than" value includes three of five samples being assumed present at detection limit of 5 mg/l although they were not detectable at this limit.

(B) Cadmium not detected at indicated level of detection

Table 3. AQUEOUS PHASE CHEMICAL DATA
IOWA ARMY AMMUNITION PLANT OCTOBER 1975
BRUSH CREEK STATIONS - MEANS

Parameter	Units	B1	B2	B3	B4	B5	B6	B7	B8
Specific Conductance	µmhos/cm	1500	1040	1100	1060	1200	950	640	700
Total Solids	mg/l	1480	789	776	724	854	711	469	486
Total Suspended Solids	mg/l	40	17	5	2	7	7	46	3
pH	SU*	6.60	9.35	9.25	9.35	8.60	8.40	8.20	8.10
Total Alkalinity	mg/l as $CaCO_3$	146	153	159	162	153	160	135	155
Chloride	mg/l	25.7	241	234	258	353	209	109	136
Sulfate	mg/l	870	98	100	100	96	96	67	77
Total Hardness	mg/l as $CaCO_3$	890	229	213	204	236	205	185	223
Calcium	mg/l	200	45.1	40.7	38.2	42.0	38.6	37.4	47.2
Magnesium	gm/l	95	28.2	27.2	26.4	31.9	26.7	22.4	25.7
Sodium	mg/l	34	172	176	170	187	142	75	86
Potassium	mg/l	3.4	10.6	10.2	10.6	11.6	10.6	7.6	8.8
Dissolved Oxygen	mg/l	6.2	8.4	8.5	9.4	9.4	10.3	9.4	9.4
BOD1	mg/l	2	3	2	2	2	3	2	2
COD	mg/l	14	18	19	20	17	21	11	14
TOC	mg/l	6	7	8	6	7	6	6	5
Kjeldahl-N	mg/l	0.8	0.9	0.8	0.8	0.7	0.7	0.9	0.7
Ammonia-N	mg/l	0.44	0.24	0.28	0.22	0.15	0.043	0.086	0.032
Nitrite-N	mg/l	0.003	0.005	0.007	0.005	0.008	0.007	0.004	0.007
Nitrate-N	mg/l	0.092	0.40	0.30	0.31	0.45	1.1	3.1	1.5
Total Phosphorus	mg/l	0.030	0.71	0.77	0.75	1.6	3.7	2.2	1.6

Table 3 (continued)

Parameter	Units	B1	B2	B3	B4	B5	B6	B7	B8
Cadmium	mg/l	-	-	-	-	-	-	-	-
Chromium	mg/l	0.001	0.108	0.058	0.034	0.029	0.058	0.026	0.017
Iron	mg/l	-	-	-	-	-	-	-	-
Lead(A)	mg/l	0.008	0.002	<0.001 (4/5)	<0.001 (4/5)	<0.001 (4/5)	<0.001 (5/5)	<0.001 (5/5)	<0.001 (5/5)
Manganese	mg/l	-	-	-	-	-	-	-	-
Mercury	mg/l	-	-	-	-	-	-	-	-

*Median Value

(A) Number in parenthesis indicates (number of samples not detected/number of samples taken)

nitrogen were especially notable. Although no particular problems concerning BOD and dissolved oxygen were observed during the June survey, the low hydrogen ion concentrations measured during this period are considered severe modifications of the basic water quality of such a stream. Of the trace metals determined, only chromium was found to be significantly different from background conditions. The average values of 0.163, 0.122 and 0.095 mg/l at stations B3, B3 and B4, respectively are considered high by normal freshwater stream standards.

Mean values for general water quality parameters measured during the fall survey period are presented in Table 3 . The trends observed in Brush Creek during this period are virtually identical to those of the summer survey, though the magnitude of the species enrichment is somewhat different due to differing amounts of ground water runoff and industrial activity. This is especially true for the dissolved solids burden, which during the fall survey was twice as high in Brush Creek from station B2 through station B8 as compared with natural background levels. It should be noted that for this comparison stream station B1 cannot be considered an adequate control for lower Brush Creek stations since there was no flow at this upstream station during the fall sampling period.

Water samples taken during the fall survey were not analyzed for the trace metals cadmium, iron, manganese and mercury. The analyses were precluded since the concentrations of these components in the summer samplings were not especially high and could not be considered environmentally significant effects of the IAAP industrial operations on the Brush Creek stream system.

Munitions Compounds -

Average values for munitions-related compounds in the aqueous phase are presented in Tables 4 and 5 . The analysis of samples from the IAAP was tailored to provide quantitative information on 2,6-dinitrotoluene and 2,4-dinitrotoluene (both minor components in technical grade TNT),

Table 4. AQUEOUS PHASE MUNITION DATA
IOWA ARMY AMMUNITION PLANT JUNE 1975
BRUSH CREEK STATIONS - MEANS

Parameter	Units	Det. Limit	B1	B2	B3	B4	B5	B6	B7	B8
2,6-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,4-Dinitrotoluene	µg/l	0.1	<0.2	<0.1	<0.1	<0.2	0.1 (4/5)	<0.1	<0.1	0.1 (4/5)
1,3,5-Trinitoluene	µg/l	0.2	<0.6	0.2 (3/5)	<0.2	<0.2	0.4 (3/5)	<0.2	0.4 (3/5)	0.7 (3/5)
2,4,6-Trinitroluene	µg/l	0.2	<0.2	<0.2	<0.2	2.5 (1/5)	3.4 (2/5)	0.3 (2/5)	4.1 (2/5)	1.3 (3/5)
4-Hydroxylamino-2,6-Dinitrotoluene	µg/l	5	<5	6 (4/5)	<5	6 (4/5)	10 (1/5)	7 (3/5)	8 (3/5)	<5
2,Hydroxylamino-4,6-Dinitrotoluene	µg/l	10	<10	<10	<10	11 (4/5)	18 (4/5)	12 (4/5)	21 (2/5)	12 (4/5)

Note: "less than" value indicates no detectable amount at given detection limit

Note: number in parenthesis represents (number of samples with concentrations below indicated detection limit/total number of samples taken)

Table 5. AQUESOUS PHASE MUNITIONS DATA
IOWA ARMY AMMUNITION PLANT OCTOBER 1975
BRUSH CREEK STATIONS - MEANS

Parameter	Units	Det. Limit	B1	B2	B3	B4	B5	B6	B7	B8
2,6-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,4-Dinitrotoluene	µg/l	0.1	<0.1	0.1 (4/5)	0.1 (4/5)	0.1 (4/5)	<0.1	<0.1	0.1 (4/5)	<0.1
1,3.5-Trinitrobenzene	µg/l	0.2	<0.2	0.6 (4/5)	0.3 (4/5)	0.5 (4/5)	<0.2	<0.2	<0.2	<0.2
2,4,6-Trinitrotoluene	µg/l	0.2	<0.2	0.5 (2/5)	0.2 (4/5)	0.8 (0/5)	0.5 (2/5)	<0.2	0.5 (4/5)	0.3 (4/5)
4-Hydroxylamino-2,6-Dinitrotoluene	µg/l	5	<5	<5	<5	<5	<5	5 (4/5)	5 (3/5)	5 (4/5)
2-Hydroxylamino-4,6-Dinitrotoluene	µg/l	10	<10	<10	<10	<10	<10	<10	<10	<10

Note: "less than" value indicates no detectabel amount at given detection limit

Note: number in parenthesis represents (number of samples with concentration below indicated detection limit/total number of samples taken)

1,3,5-trinitrobenzene (an important photolysis product of alpha TNT), 2,4,6-trinitrotoluene (the main TNT isomer), and 4-hydroxylamino-2,6-dinitrotoluene and 2-hydroxylamino-4,6-dinitrotoluene (two known environmental transformation products of alpha TNT). Detection limit for each of the compounds were determined by the minimum amount of each compound which could be distinguished with adequate confidence from the indigenous oils present in each sample extract. For this reason, detection limits vary from compound to compound, from station to station, and occasionally from one sample to another at a given station.

During the June sampling period, munitions-related compounds were detected in the aqueous phase at stream stations B2, B4, B5, B6, B7 and B8. Sources of the munitions compounds in Brush Creek are evident from the data on IAAP industrial outfall concentrations in Tables 6 and 7 . Industrial stations 4 and 7, and to a lesser extent stations 3 and 5, appear to be the point sources responsible for the munitions-related compounds present in Brush Creek. The levels found at these outfalls are in the low microgram per liter range and probably represent the residual materials remaining in the industrial process water after passage through activated carbon treatment devices.

Samples collected during the fall survey reveal detectable quantities of munitions-related compounds at all Brush Creek stations from B2 through B8, though the average concentrations during this survey period are lower than during June. This probably results from the lower production activity of the IAAP during the fall survey period.

Sediment Phase

General Sediment Chemistry -

Characterization of the bottom sediment deposits is an important part of any aquatic survey. Not only does the sediment chemistry significantly affect the biota normally associated with bottom deposits, but in a chemical sense the sediments can serve as a source or sink for constituents

Table 6 . AQUEOUS PHASE MUNITIONS DATA
IOWA ARMY AMMUNITION PLANT JUNE 1975
INDUSTRIAL STATIONS - MEANS

Parameter	Units	Det. Limit	Station I1	I2	I3	I4	I5	I7
2,6-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,4-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	0.2(3/5)	<0.1	0.2(1/5)	0.2(3/5)
1,3,5-Trinitrobenzene	µg/l	0.2	<0.2	<0.2	0.8(3/5)	<0.2	0.5(2/5)	0.3(3/5)
2,4,6-Trinitrotoluene	µg/l	0.2	<0.2	<0.2	0.5(3/5)	11.7(0/5)	0.4(4/5)	3.4(1/5)
4-Hydroxylamino-2,6-Dinitrotoluene	µg/l	Variable	<6	<5	6 (4/5)	5 (3/5)	23 (2/5)	7 (3/5)
2-Hydroxylamino-4,6-Dinitrotoluene	µg/l	10	<10	<10	10 (4/5)	10 (4/5)	32 (3/5)	11 (4/5)

Note: "less than" value indicates no detectable amount at given detection limit

Note: number in parenthesis represents (number of samples with concentration below indicated detection limit/total number of samples taken)

Table 7. AQUEOUS PHASE MUNITIONS DATA
IOWA ARMY AMMUNITION PLANT OCTOBER 1975
INDUSTRIAL STATIONS - MEANS

Parameter	Units	Det. Limit	Station I1	I2	I3	I4	I5	I7
2,6-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
2,4-Dinitrotoluene	µg/l	0.1	<0.1	<0.1	<0.1	0.2 (4/5)	<0.1	<0.1
1,3,5-Trinitrobenzene	µg/l	0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
2,4,6-Trinitrotoluene	µg/l	0.2	<0.2	<0.2	<0.2	16.7 (1/5)	<0.2	6.0 (1/5)
4-Hydroxylamino-2,6-Dinitrotoluene	µg/l	5	<5	<5	7 (0/5)	5 (2/5)	<5	11 (3/5)
2-Hydroxylamino-4,6-Dinitrotoluene	µg/l	10	<10	<10	14 (2/5)	14 (2/5)	<10	20 (3/5)

Note: "less than" value indicates no detectable aount at given detection limit

Note: number in parenthesis represents (number of samples wth concentration below indicated detection limit/total number of samples taken)

found in the aqueous phase. At the IAAP three core samples were collected at each stream station during each survey period. In general, these core samples can be considered replicates since they were taken within a single sediment formation and usually were withdrawn within a one half meter radius. However, at certain stations several sediment types could be found, and in an attempt to obtain sediment characteristics representative of the general sampling area, cores were taken in one or more of the sediment formations present. The variation about the mean for samples from such a station will be predictably greater than for those cores taken from a station where only one major sediment formation exists. For this reason, the physical descriptions of sediment cores listed in Tables 8 through 11 are important considerations when comparing the sediment chemistry of one station with another, or even whem comparing "replicate" core samples taken at a given station.

Mean values for general sediment chemistry parameters are presented in Table 12 for the June survey period, and Table 13 for the October sampling. In comparing the concentrations of various parameters in Brush Creek with background levels normally anticipated for such streams, it is not completely justifiable to use stream station B1 as a control. This is due to the fact that the sediment at B1 can be accurately described as rich topsoil. Such a bottom material is uncharacteristic of continuously flowing streams. Rather, a drainage ditch with low and intermittent flow is suggested. The bottom deposits of such drainage ditches collect excessive amounts of nutrient-rich silt.

Sediments from stations B2, B3 and B7 show the most significant increases above background levels during the June survey. Elevated levels of hexane extractables, Kjeldahl-nitrogen, total phosphorus, chromium and mercury observed at station B7 can be attributed to a large extent on the effluent discharged from the IAAP domestic wastewater plant. The mean concentration of mercury here is particularly noteworthy. Increases in most of the general sediment chemistry parameters observed at stations B2 and B4 can be attributed to the activities associated with IAAP pro-

Table 8. SEDIMENT DESCRIPTION
IOWA ARMY AMMUNITION PLANT 25 JUNE 1975
BRUSH CREEK STATIONS

Sample	Sampling Device	Sediment Depth	Color	Fraction >841 um	Description
B1-1	Corer	0-10 cm	Dark Brown	3.6%	Soil
B1-2	Corer	0-10 cm	Black	1.8%	Soil
B1-3	Corer	0-10 cm	Black	2.3%	Soil
	Corer	10-16 cm	Black	0.0%	Soil
B2-1	Corer	0-10 cm	Brown	41.4%	Sand, stones and coal fragments
	Corer	10-20 cm	Brown	33.7%	Sand, gravel and detritus
	Corer	20-28 cm	Brown	36.2%	Sand, clay and some detritus
B2-2	Corer	0-10 cm	Brown	47.9%	Sand
	Corer	10-20 cm	Brown	14.3%	Sand, silt and detritus
B2-3	Corer	0-10 cm	Brown	25.1%	Gravel with detritus
	Corer	10-20 cm	Dark Brown	2.0%	Sand overlying detritus
	Corer	20-30 cm	Brown	2.5%	Clay with detritus
B3-1	Corer	0-10 cm	Brown	29.2%	Coarse sand overlying clay;stones
B3-2	Corer	0-10 cm	Brown	41.5%	Gravel with clay
B3-3	Corer	0-10 cm	Brown	12.0%	Coarse sand
B4-1	Corer	0-10 cm	Brown	18.6%	Coarse sand with detritus
	Corer	10-18 cm	Brown	35.1%	Sand, clay, stones and detritus
B4-2	Corer	0-10 cm	Brown	11.9%	Ooze with detritus
	Corer	10-20 cm	Brown	14.2%	Sand, ooze and detritus
	Corer	20-26 cm	Brown	21.4%	Sand and ooze

Table 8. (continued).

Sample	Sampling Device	Sediment Depth	Color	Fraction > 841 µm	Description
B4-3	Corer	0-10 cm	Dark Brown	7.7%	Ooze with detritus
	Corer	10-20 cm	Dark Brown	1.9%	Sand and clay with detritus
B5-1	Corer	0-10 cm	Brown	22.7%	Coarse sand
	Corer	10-20 cm	Brown and Gray	49.4%	Coarse sand, clay, stones and detritus
	Corer	20-30 cm	Brown	0.2%	Sand with clay
B5-2	Corer	0-10 cm	Brown	19.3%	Sand
	Corer	10-20 cm	Brown and Gray	38.4%	Sand with clay; stones
	Corer	20-30 cm	Gray	6.6%	Clay
B5-3	Corer	0-10 cm	Brown	22.7%	Sand, clay and detritus
	Corer	10-20 cm	Brown	40.2%	Sand with detritus
	Corer	20-28 cm	Brown	37.4%	Sand with detritus
B6-1	Corer	0-10 cm	Brown	23.1%	Coarse sand
	Corer	10-20 cm	Brown	3.5%	Sand
B6-2	Corer	0-10 cm	Brown	26.9%	Coarse sand
	Corer	10-20 cm	Brown	3.7%	Sand with clay
	Corer	20-30 cm	Brown	0.0%	Coarse sand with clay
B6-3	Corer	0-10 cm	Brown	19.6%	Coarse sand
	Corer	10-20 cm	Brown	12.2%	Coarse sand and clay
	Corer	20-28 cm	Gray	0.0%	Clay
B7-1	Corer	0-10 cm	Brown	6.6%	Sand
	Corer	10-19 cm	Brown	25.0%	Coarse sand with stones

Table 8. (continued).

Sample	Sampling Device	Sediment Depth	Color	Fraction >841 µm	Description
B7-2	Corer	0-10 cm	Brown	14.4%	Coarse Sand
	Corer	10-20 cm	Brown	19.0%	Coarse sand, stones and detritus
	Corer	20-30 cm	Brown	42.9%	Coarse sand and detritus
B7-3	Corer	0-10 cm	Brown	19.1%	Sand
	Corer	10-20 cm	Brown	43.4%	Sand with large stones
	Corer	20-26 cm	Brown	33.2%	Sand with detritus
B8-1	Corer	0-10 cm	Brown	32.4%	Coarse sand, clay and stones
B8-2	Corer	0-9 cm	Brown	15.6%	Coarse sand and clay
B8-3	Corer	0-10 cm	Brown	44.0%	5 cm gravel overlying 5 cm clay

Table 9. SEDIMENT DESCRIPTION
IOWA ARMY AMMUNITION PLANT 25 JUNE 1975
SPRING CREEK STATIONS

Sample	Sampling Device	Sediment Depth	Color	Fraction > 841 µm	Description
S1-1	Corer	0-6 cm	Brown	27.5%	Sand
S1-2	Corer	0-10 cm	Brown	32.7	Coarse sand with stones
S1-3	Corer	0-10 cm	Brown	16.5	Sand
S2-1	Corer	0-10 cm	Brown	18.8	Coarse sand

Table 10. SEDIMENT DESCRIPTION
IOWA ARMY AMMUNITION PLANT 15 OCTOBER 1975
BRUSH CREEK STATIONS

Sample	Samling Device	Sediment Depth	Color	Fraction > 841 µm	Description
B1-1	Corer	0-10 cm	Black	17.8%	Soil with detritus
B1-2	Corer	0-10 cm	Black	0.4%	Soil
B1-3	Corer	0-10 cm	Black	4.6%	Soil
B2-1	Corer	0-10 cm	Dark Brown	42.4%	Coarse sand with detritus
	Corer	10-20 cm	Dark Brown	46.7%	Coarse sand, clay and detritus
	Corer	20-27 cm	Brown and Gray	5.2%	Clay
B2-2	Corer	0-10 cm	Brown	37.4%	Sand with detritus and coal
	Corer	10-20 cm	Brown	44.3%	Coarse sand
	Corer	20-33 cm	Light Brown	17.1%	Coarse sand overlying clay
B2-3	Corer	0-10 cm	Dark Brown	43.5%	Coarse snad with detritus
	Corer	10-20 cm	Dark Brown	19.2%	Coarse sand
	Corer	20-27 cm	Brown	33.6%	Coarse sand with clay
B3-1	Corer	0-10 cm	Dark Brown	18.2%	Detritus with fine sand
	Corer	10-20 cm	Brown	60.4%	Coarse gravel, sand and clay
	Corer	20-30 cm	Light Brown	46.1%	Gravel with coarse sand
B3-2	Corer	0-10 cm	Gray	50.9%	5 cm sand overlying 5 cm clay; stones
	Corer	10-20 cm	Gray	41.5%	Coarse sand
	Corer	20-30 cm	Gray	21.0%	Clay with detritus

Table 10.(continued).

Sample	Sampling Device	Sediment Depth	Color	Fraction > 841 µm	Description
B3-3	Corer	0-10 cm	Dark Brown	10.3%	Sand with detritus
	Corer	10-20 cm	Brown	42.0%	Sand, clay and detritus
	Corer	20-26 cm	Brown	67.3%	Gravel
B4-1	Corer	0-10 cm	Brown and green	19.8%	Sand
	Corer	10-20 cm	Gray	8.8%	Clay
	Corer	20-26 cm	Brown	37.4%	Coarse sand
B4-2	Corer	0-10 cm	Brown	33.6%	Coarse sand
	Corer	10-18 cm	Brown	50.2%	Coarse sand with large rocks, some coal
B4-3	Corer	0-10 cm	Brown and green	21.9%	Gravel and clay
	Corer	10-20 cm	Brown and green	12.9%	Clay
	Corer	20-30 cm	Brown	44.1%	Coarse sand
B5-1	Corer	0-10 cm	Brown	19.6%	Sand with some coal
	Corer	10-20 cm	Brown and gray	17.0%	5 cm sand overlying 5 cm clay
	Corer	20-25 cm	Light Gray	0.0%	Clay
B5-2	Corer	0-10 cm	Brown	13.4%	Coarse sand with clay
	Corer	10-20 cm	Brown	11.1%	Coarse sand with clay
	Corer	20-30 cm	Brown and gray	0.1%	Clay
B5-3	Corer	0-10 cm	Brown	19.2%	Sand
	Corer	10-20 cm	Gray	25.6%	Clay

Table 10. (continued).

Sample	Sampling Divice	Sediment Depth	Color	Fraction >841 µm	Description
B6-1	Corer	0-10 cm	Brown	38.6%	Coarse sand with gravel
	Corer	10-20 cm	Dark Brown	52.9%	Gravel, sand and coal
B6-2	Corer	0-10 cm	Vary	49.1%	Coarse sand with detritus
	Corer	10-20 cm	Gray	7.2%	Clay with detritus
	Corer	20-26 cm	Gray and Brown	19.8%	Clay with detritus
B6-3	Corer	0-10 cm	Brown	47.6%	Coarse sand with some coal
	Corer	10-20 cm	Brown	44.5%	Coarse sand with some coal
	Corer	20-24 cm	Gray	6.9%	Ooze with detritus
B7-1	Corer	0-10 cm	Dark Brown	25.5%	Sand and silt
	Corer	10-20 cm	Dark Brown	7.3%	Silt and clay
B7-2	Corer	0-10 cm	Dark Brown	18.9%	Sand overlying ooze
	Corer	10-21 cm	Dark Brown	4.5%	Interspersed layers of sand and ooze
B7-3	Corer	0-10 cm	Brown	17.8%	Sand
	Corer	10-20 cm	Brown and Gray	10.4%	Sand and clay
B8-1	Corer	0-10 cm	Brown	20.0%	Sand with detritus
	Corer	10-14 cm	Brown	15.2%	Coarse sand
B8-2	Corer	0-10 cm	Brown	14.5%	Ooze overlying sand
	Corer	10-21 cm	Brown	20.6%	Sand with some coal
B8-3	Corer	0-10 cm	Brown	9.2%	Coarse sand
	Corer	10-16 cm	Brown	15.2%	Coarse sand

Table 11. SEDIMENT DESCRIPTION
IOWA ARMY AMMUNITION PLANT 15 OCTOBER 1975
SPRING CREEK STATIONS

Sample	Sampling Device	Sediment Depth	Color	Fraction > 841 µm	Description
S1-1	Corer	0-10 cm	Black	3.8%	Silt with detritus
	Corer	10-15 cm	Black	1.0%	Silt with detritus
S1-2	Corer	0-10 cm	Gray	30.5%	Coarse sand
	Corer	10-18 cm	Gray to Brown	52.2%	Sand
S1-3	Corer	6-10 cm	Brown	38.0%	Sand
S2-1	Corer	0-5 cm	Brown	33.4%	Coarse sand and gravel
S2-2	Corer	0-10 cm	Brown	11.7%	Sand
S2-3	Corer	0-10 cm	Brown	28.4%	Sand with detritus

Table 12. IOWA ARMY AMMUNITION PLANT
SEDIMENT PHASE CHEMICAL DATA
BRUSH CREEK STATIONS: 0-10 cm SECTION MEANS
25 June 1975

Parameter	Units	Station B1	B2	B3	B4	B5	B6	B7	B8
Total Solids	%	74.3	82.2	82.2	75.9	84.6	84.1	82.7	82.0
Total Volatile Solids	% dry weight	10.1	10.8	3.2	6.3	2.1	1.6	2.4	3.3
COD	mg/g	70	110	16	44	20	12	27	8
Hexane Extractables	mg/kg	370	910	160	310	270	130	220	180
Kjeldahl-N	mg/kg	1660	1450	330	970	300	190	430	230
Nitrate + Nitrite-N	mg/kg	440	520	410	500	280	320	290	200
Total Phosphorus	mg/kg	750	960	1030	780	600	530	610	740
Cadmium	mg/kg	1	1	1	1	1	<1	1	1
Chromium	mg/kg	11.6	20.7	12.9	31.3	17.8	35.3	43.7	14.3
Iron	mg/g	11.6	16.3	12.6	11.9	7.2	6.8	6.3	8.2
Mercury	mg/kg	0.04	0.10	0.06	0.15	0.09	0.11	4.54	0.03
Manganese	mg/kg	1140	1370	1150	1310	680	980	1050	590
Lead	mg/kg	21	25	16	30	16	15	19	23

Table 13. IOWA ARMY AMMUNITION PLANT
SEDIMENT PHASE CHEMICAL DATA
BRUSH CREEK STATIONS: 0-10 cm SECTION MEANS
15 October 1975

Parameter	Units	Station B1	B2	B3	B4	B5	B6	B7	B8
Total Solids	%	72.8	77.1	76.5	71.6	82.9	80.1	80.8	80.2
Total Volatile Solids	% dry weight	9.1	7.5	3.8	8.4	1.3	3.2	3.3	2.7
COD	mg/g	47	64	29	79	9	19	13	15
Hexane Extractables	mg/kg	140	170	270	420	130	220	130	200
Kjeldahl-N	mg/kg	1580	990	660	1560	220	300	560	410
Nitrate + Nitrite-N	mg/kg	160	170	210	160	130	170	140	170
Total Phosphorus	mg/kg	710	760	720	990	290	710	400	360
Cadmium	mg/kg	1	<1	<1	1	<1	< 1	1	1
Chromium	mg/kg	10.7	26.0	26.7	54.5	11.8	55.1	7.4	15.9
Iron	mg/g	11.7	12.6	8.0	10.9	4.1	7.3	6.5	5.4
Mercury	mg/kg	0.03	0.08	0.09	0.09	0.03	0.02	0.24	0.03
Manganese	mg/kg	1170	830	370	580	420	670	320	510
Lead	mg/kg	23	23	16	19	6	16	8	10

duction lines. These increases involve primarily nitrogen, phosphorus and organic carbon concentrations. Certain metals, including chromium, iron, mercury and lead, are also enriched as a result of these activities.

The sediment samples collected during October also reflect this trend of enrichment, with stations B2 and B4 generally showing the greatest increases in those general sediment chemistry parameters monitored. Similarly, core samples from station B8 taken during both summer and fall surveys reveal that the general sediment quality improves to near background conditions as the stream descends to the IAAP boundary.

Munitions Compounds -

Average concentrations for munitions-related compounds in the IAAP sediments are presented in Tables 14 and 15 . A review of the summer survey data reveals that detectable quantities of munitions compounds and specific transformation products were found at stations B2, B4, B5, B6 and B7. As during the 1974 survey period, station B4 contained the greatest quantities of 2,4,6-trinitrotoluene of all Brush Creek stations. Station B2, which was the intended control station for the 1974 survey, was also found to contain significant concentrations of TNT and transformation products, presumably originating from industrial outfalls 3 and 4. It is interesting to note that no munitions-related compounds were detected in sediments from stream station B8 collected during the 1975 summer survey.

Analysis of sediments collected in October revealed munitions-related compounds at stations B2, B4, B5, B6, B7 and B8. Again during this survey, station B4 had the highest average 2,4,6-TNT concentration of any stream sediment sampled. Indeed, one core from this station contained over 200 mg/kg of alpha TNT. Sediment concentrations of all munitions-related compounds declined downstream of B4, though during the fall survey some residue was still detectable at station B8.

During the summer survey, an additional source of munitions-related compounds was discovered along Brush Creek. Situated on the east side of

Table 14. SEDIMENT PHASE MUNITIONS DATA
IOWA ARMY AMMUNITION PLANT 25 JUNE 1975
BRUSH CREEK STATIONS: 0-10 cm SECTION MEANS

Parameter	Units	B1	B2	B3	B4	B5	B6	B7	B8
2,6-Dinitrotoluene	mg/kg	<0.1	2.4	< 0.1	< 0.1	< 0.1	< 0.1	0.1	<0.1
2,4-Dinitrotoluene	mg/kg	< 0.4	<0.1	< 0.1	0.1	< 0.1	0.1	0.2	<0.1
1,3,5-Trinitrobenzene	mg/kg	<1.3	3.5	<1.0	1.4	<1.0	1.2	<1.0	<1.0
2,4,6-Trinitrotoluene	mg/kg	<1.0	9.0	< 0.2	18.7	0.3	1.0	2.7	<0.2
4-Hydroxylamino-2,6-Dinitrotoluene	mg/kg	< 5	8	< 5	9	<5	<5	<5	<5
2-Hydroxylamino-4,6-Dinitrotoluene	mg/kg	<30	90	<30	45	<30	<30	<30	<30

Note: "less than" value indicates material not detected - detection limits indicated in Appendix of Volume 1.

Table 15 . IOWA ARMY AMMUNITION PLANT
SEDIMENT PHASE MUNITIONS DATA
BRUSH CREEK STATIONS: 0-10 cm SECTION MEANS
15 OCTOBER 1975

Parameter	Units	Station B1	B2	B3	B4	B5	B6	B7	B8
2,6-Dinitrotoluene	mg/kg	<0.2	0.3	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
2,4-Dinitrotoluene	mg/kg	<0.3	<0.1	<0.1	0.9	0.1	0.3	0.1	<0.1
1,3,5-Trinitrobenzene	mg/kg	<3.7	1.6	<1.0	2.0	<1.0	5.1	<1.0	1.3
2,4,6-Trinitrotoluene	mg/kg	<0.2	2.6	<0.2	111	2.0	2.0	0.3	0.4
4-Hydroxylamino 2,6-Dinitrotoluene	mg/kg	<5	<5	<5	57	<5	<5	<5	<5
2-Hydroxylamino-4,6-Dinitrotoluene	mg/kg	<43	44	<30	101	33	<30	<30	<30

the stream, approximately 950 meters downstream of B3 and 250 meters upstream of B4, a large vegetation-free plateau was found. The entire plateau was highly eroded and the barren soil was reddish in color. Plant personnel were queried as to the history of this area and it was learned that it was previously the site of a "pink-water" treatment lagoon. Process water from the TNT operations of Group 1 was collected in this lagoon, which actually was a retention pond in Brusn Creek itself. Since the wastewater was acidic, flyash from the then coal-burning power plant was also dumped into the lagoon to help neutralize the acidic wastes. Considerable amounts of solid coal-wastes were dumped into the lagoon with the idea that a certain amount of carbon adsorption of munitions-related compounds would be attained and these adsorbed materials would be thus transported from the aqueous phase to the bottom sediments. Spill-over from the lagoon simply continued down Brush Creek to another treatment installation located under the bridge in Road H near the IAAP sewage disposal plant, where excess acidity was neutralized with the addition of base.

According to contractor personnel, the lagoon was allowed to go into a state of disrepair and has not been used for approximately 20 years. This date conincides with the initial use of physiochemical waste treatment on munitions-bearing wastes at the IAAP. Given the long period of time since large quantities of TNT were added to the lagoon, it is somewhat surprising to find over 3000 mg/kg of the highly reactive 2,4,6-isomer present in soil there today. A surface-scraping sample of this barren soil was collected during the June survey and analyzed only for munitions-related compounds. The results are presented in Table 16. Soil from this barren area, dubbed "TNT Flats" by the field survey crew, contains large amounts of the hydroxylamine transformation products in addition to 2,4,6-TNT.

It was observed during the 1974 survey and during both 1975 surveys that after a rainfall, small puddles of "pink-water" would collect at high water areas of downstream stations in Brush Creek, especially at station B4. Given the denuded and highly eroded physical nature of the "TNT Flats" area, it seems likely that leachate from this area is responsible for

Table 16. SEDIMENT PHASE MUNITIONS DATA
FROM "TNT FLATS" NEAR
IOWA ARMY AMMUNITION PLANT
STATION B4

Parameter	Concentration (mg/kg)
2,6-Dinitrotoluene	0.5
2,4-Dinitrotoluene	3.0
1,3,5-Trinitrobenzene	0.6
2,4,6-Trinitrotoluene	3030
4-Hydroxylamino-2,6-Dinitrotoluene	101
2-Hydroxylamino-4,6-Dinitrotoluene	180

these puddles. Further, since the sediments at station B5 do not show the same level of munitions compounds enrichment as those at B4, despite the fact that Group 2 is perhaps the busiest conventional munitions processing operation along Brush Creek, it seems likely that leachate from the "TNT Flats" area is responsible for the very high concentrations of munitions-related compounds observed in the B4 sediments. Indeed, this leachate should probably be considered the significant source of such compounds throughout the downstream areas of Brush Creek. When the carbon treatment systems are functioning properly at each of the munitions processing installations along Brush Creek, it is quite possible that leachate from the "TNT Flats" may be the major source of trinitrotoluene and its transformation products in the Brush Creek system. Analysis of the quality and quantity of leachate from this area during dry weather and storm events would allow the evaluation of its impact on Brush Creek.

Another area of very high concentrations of munitions compounds was found near the IAAP Group 800. A large impoundment of "pink-water", used primarily as a wastewater storage area, is situated just east of the Group 800 security fence. Sediment samples taken from this impoundment contain approximately the same level of munitions related compounds as soil samples from the "TNT Flats". A breach in the diking system around this impoundment during 1974 may well be responsible for "pink-water" puddles observed at downstream Brush Creek stations during the 1974 field survey. The diking system has been rebuilt and was intact throughout both 1975 survey periods. Conflicting stories were obtained from IAAP personnel concerning the present utilization of this impoundment.

In order to summarize the effects of IAAP production lines on the level of munitions-related compounds in the Brush Creek system, average concentrations found in the aqueous and sediment phases during the summer and fall surveys are found in Tables 14 and 15, respectively.

RESULTS AND DISCUSSION - JAAP

Aqueous Phase

General Water Quality -

Analytical results of the single set of samples collected during June at Joliet

AAP are presented in Table 17. In reviewing the table, it is apparent that the industrial wastes discharged into the TNT ditch have a very significant impact on the water quality of Grant Creek immediately below the point of confluence of the two streams. Specific conductance and the level of dissolved solids are both about 13 percent higher at G2 than at control station G1. The difference in dissolved solids can be accounted for by increased in chloride, sulfate, magnesium, sodium potassium and nitrate-nitrogen. A drop in tital organic carbon was observed as the stream descended from G1 to G2. Other environmentally significant increases were found in COD, ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen. The nitrate concentration in Grant Creek above the munitions production area is very high by normal freshwater stream standards. However, this background level of 8.4 mg/1 at station G1 was raised to 13 mg/1 at G2 by the addition of nitrate-bearing wastes from the TNT ditch.

The water sampling on 2 June 1975 revealed that facilities at JAAP were responsible for at least one very significant acid wastewater discharge during the three days of the survey. The data for station T2 in Table 17 indicates that nitric acid, and possibly some sulfuric, was discharged to the TNT ditch in sufficient quantities to completely break the chemical buffer capacity and depress the pH to 2.51. This discharge was apparently confined to a narrow slug since attempts to pinpoint the source by backtracking upstream with pH monitoring equipment immediately after discovering the acidic condition at T2 was unsuccessful in locating any other significant pH depressions along the ditch.

The general water chemistry in TNT ditch, even without intermittent acid discharges, appears to be quite different from that of Grant Creek. While the level of total dissolved solids is approximately the same, the concentration distirbution of these solutes is different. In addition, all forms of nitrogen measured were found to be significantly higher at the two TNT ditch stations and thus flow from the ditch must be considered a detrimental source of biostimulatnts for the lower reaches of Grant Creek.

Table 17. JOLIET ARMY AMMUNITION PLANT

Aqueous Phase Chemical Data
Grant Creek and TNT Ditch Stations
2 June 1975

Parameter	Units	Station G1	G2	T1	T2
Specific Conductance	µmhos/cm	710	800	720	3550
Total Solids	mg/l	502	563	492	789
Total Suspended Solids	mg/l	31	30	32	14
pH	SU	8.18	7.90	7.90	2.51
Total Alkalinity	mg/l as $CaCO_3$	228	211	151	0
Chloride	mg/l	30.1	36.7	27.8	26.2
Sulfate	mg/l	94	120	120	250
Total Hardness	mg/l as $CaCO_3$	312	319	225	664
Calcium	mg/l	72.2	72	47.5	141
Magnesium	mg/l	32	34	26	76
Sodium	mg/l	12.8	24.2	48.3	120
Potassium	mg/l	3.0	4.1	4.8	7.7
Dissolved Oxygen	mg/l	9.7	9.2	7.6	6.9
BOD	mg/l	1	2	5	3
COD	mg/l	<5	11	15	49
TOC	mg/l	14	11	10	17
Kjeldahl-N	mg/l	0.3	0.5	1.9	0.1
Ammonia-N	mg/l	0.096	0.37	1.4	1.2
Nitrite-N	mg/l	0.069	0.18	0.58	0.52
Nitrate-N	mg/l	8.4	13	15	260
Total Phosphorus	mg/l	0.11	0.093	0.090	0.14

Table 17 (continued).

Parameter	Units	Station G1	G2	T1	T2
Cadmium	mg/l	0.0002	0.0003	0.0001	0.0014
Chromium	mg/l	0.002	0.002	0.007	0.008
Iron	mg/l	0.90	0.87	1.40	4.24
Lead	mg/l	<0.001	0.002	0.013	0.31
Manganese	mg/l	0.040	0.081	0.195	0.824
Mercury	mg/l	<0.0001	< 0.0001	0.0002	0.0001

Note: "less than" sign indicates parameter not detected at indicated detection limit

Munition Compounds -

A summary of the aqueous phase munitions compounds present in Grant Creek and the TNT ditch appears as Table 18. As evident from this data, the levels of munitions-related compounds in the ditch are quite high, in some cases exceeding several parts per million for the primary munitions compounds. These elevated concentrations result in significant levels of munitions-related compounds in Grant Creek at station G2. Alpha TNT and the 2,6- and 2,4-dinitrotoluene isomers constitute the major species occurring in at this point in Grant Creek, although all munitions-related compounds under study, with the exception of 2,4,6-trinitrobenzaldehyde were found here. As in the case of other aqueous constituents, the sludge flow loadings to Grant Creek are apparent from the aqueous munitions data.

In general, the level of munitions compounds found in the aqueous phase at JAAP were two to three orders of magnitude higher than those concentrations observed at the Iowa AAP.

Sediment Phase

Sediment concentrations referred to in this report are, in general, mean values of results obtained from the analysis of more than one core sample. These averages are reported in tables within the body of this report. Discrete values, as well as statistical information concerning scatter about the mean, are presented as appendicies to this report.

General Sediment Chemistry -

The physical characteristics of sediments collected at JAAP are delineated in Table 19. An important observation to be made from this table concerns the extreme difference in sediment types found at Grant Creek stations G1 and G2. Whereas the bottom deposits at G1 consisted primarily of highly organic silt, the sediments at station G2 were virtually entirely sand. This difference evidences itself in the general sediment chemistry at each of the two locations, as seen in Table 20.

The small particle size coupled with the highly organic character of sediments at station G1 result in higher sediment concentrations of carbon,

Table 18. AQUEOUS PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 2 JUNE 1975
GRANT CREEK AND TNT DITCH STATIONS

Parameter	Units	Station G1	G2	T1	T2
2,6-Dinitrotoluene	µg/l	< 0.1	51	380	3550
2,4-Dinitrotoluene	µg/l	< 0.1	320	1370	7380
1,3,5-Trinitrobenzene	µg/l	< 0.2	16	97	76
2,4,6-Trinitrotoluene	µg/l	< 0.2	86	430	1020
2,4,6-Trinitrobenzaldehyde	µg/l	< 0.2	< 0.2	< 0.2	3.3
4-Hydroxylamino-2,6-Dintrotoluene	µg/l	< 1	5	25	11
2-Hydroxylamino-4,6-Dintrotoluene	µg/l	< 1	3	42	10

Note: "less than" sign indicates parameter not detected at indicated detection limit

Table 19. SEDIMENT DESCRIPTION
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK AND TNT DITCH STATIONS

Sample	Sampling Device	Sediment Depth	Color	Fraction > 841 um	Description
T1-C	Corer	0-10 cm	Vary	26.2%	Silt with sand and gravel
	Corer	10-15 cm	Brown	22.9%	Silt with gravel-sweet smell
T1-Eh	Corer	0-10 cm	Vary	5.0%	Silt with sand-sweet smell
	Corer	10-18 cm	Dark Brown	50.0%	Sand and gravel-sweet smell
T1-AEh	Corer	0-10 cm	Dark Brown	4.7%	Ooze
	Corer	10-20 cm	Dark Brown	33.5%	Ooze with detritus
T2-C	Corer	0-10 cm	Dark Brown	3.0%	Ooze with detritus
	Corer	10-20 cm	Dark Brown	0.2%	Ooze with detritus
T2-Eh	Corer	0-10 cm	Dark Brown	10.9%	Ooze with detritus
	Corer	10-20 cm	Dark Brown	1.4%	Ooze
G1-C	Corer	0-10 cm	Brown	6.5%	Silt with sand
	Corer	10-20 cm	Brown	45.0%	Silt with gravel and roots
	Corer	20-30 cm	Brown	54.5%	Gravel and roots
G2-C	Corer	0-10 cm	Brown	32.6%	Coarse sand
	Corer	10-20 com	Brown	19.8%	Sand
G2-Eh	Corer	0-10 cm	Brown	21.6%	Sand
	Corer	10-20 cm	Brown	15.1%	Sand with detritus
	Corer	20-25 cm	Brown	51.8%	Coarse sand with detritus

Table 20. SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK AND TNT DITCH STATIONS: 0-10 cm SECTION MEANS

Parameter	Units	G1	G2	T1	T1A	T2
Total Solids	% dry weight	72.7	81.4	74.3	60.3	58.1
Total Volatile Solids	%	6.2	2.0	4.6	21.5	8.6
COD	mg/g	33	5	37	247	67
Hexane Extractables	mg/kg	190	160	800	4950	560
Kjeldahl-N	mg/kg	1410	270	1430	9820	1830
Nitrate+Nitrite-N	mg/kg	300	90	190	260	230
Total Phosphorus	mg/kg	1010	460	770	920	830
Cadmium	mg/kg	1	2	1	1	2
Chromium	mg/kg	6.2	6.4	11.4	14.8	8.9
Iron	mg/kg	13.3	5.4	9.2	15.8	14.1
Mercury	mg/kg	0.06	0.04	0.33	0.51	0.22
Manganese	mg/kg	940	260	220	230	500
Lead	mg/kg	38	29	155	984	89

nitrogen, phosphorus and most metals than would be found in sandy deposits. This in effect means that the sediments at station G1 cannot be compared directly to those at G2 in order to evaluate the impact of effluent from the TNT ditch on bottom deposits in Grant Creek. Instead, sediment constituents at station G2 must be compared to average background values in natural sediments of similar physical character. When this is done, only the phosphorus content at G2 appears to be enriched.

It should be noted here that an attempt was made during the June survey to find stream sediments in the general area of station G2 which were finer in texture and more organic in content. Such sediments would be much more likely to accumulate TNT and other munitions-related compounds than the shifting coarse sands found in the sampling zone at station G2. This attempt was not successful.

Sediments found at stations T1, T2 and T1A were enriched in most of the constituents monitored. This is particularly true of hexane extractables, Kjeldahl-nitrogen, total phosphorus, chromium, mercury, and lead. The sediment core taken at station T1A revealed extraordinarily high levels of total volatile solids, COD, Kjeldahl-nitrogen and lead. Hexane extractables in this core were also very high, though this was undoubtedly due to the high concentration of 2,4,6-TNT in these sediments. Alpha TNT is slightly soluble in hot hexane and consequently is partially extracted along with the grease and oil substances normally measured in this determination. It suffices to say that, in general, the sediments at each of these three stations are exemplary of soils highly polluted by long term industrial waste discharges.

Munitions Compounds -

The results of analyses for munitions-related compounds in JAAP sediments are presented in Table 21 . As predicted, very little munitions residue was observed in sediments at Grant Creek station G2. Surprisingly, only minor amounts of the munitions-related compounds analyzed for were evident in sediments from station T2. This, despite the very high concentrations of these compounds which are evidently present on a routine basis in the

Table 21. SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK AND TNT DITCH STATIONS: 0-10 cm SECTION MEANS

Parameter	Units	Station G1	G2	T1	T1A	T2
2,6-Dinitrotoluene	mg/kg	< 1	<1	103	6	<1
2,4-Dinitrotoluene	mg/kg	< 1	<1	344	642	<1
1,3,5-Trinitrobenzene	mg/kg	<1	<1	<1	3	<1
2,4,6-Trinitrotoluene	mg/kg	<1	1	338	44200	3
4-Hydroxylamino-2,6-Dinitrotoluene	mg/kg	<5	<5	36	154	10
2-Hydroxylamino-4,6-Dinitrotoluene	mg/kg	< 30	<30	80	140	<30

Note: "less than" sign indicates parameter not detected at indicated detection limit

aqueous phase at this station. The low sediment levels may be related to relatively low redox potentials measured in cores from T2. This relationship will be discussed later in this section.

Sediments from station T1 contained high concentrations of all the munitions-related compounds under study except 1,3,5-trinitrobenzene. The concentration of 2,4,6-TNT at this station was approximately the same as levels of this compound observed at Brush Creek station B4 in the Iowa AAP. This is also true for the two hydroxylamine transformation products under study. The concentrations of the dinitrotoluene isomers, however, are two orders of magnitude higher at T1 than corresponding concentrations at IAAP station B4. This doubtless reflects the difference in wastewater between a plant where TNT is synthesized and a plant where TNT is processed in the assembly of munitions.

The sediment core extracted from the bottom of the red water pond at station T1A contained significant quantities of the six munitions-related compounds under study. An exceptionally large amount of alpha TNT was measured here - 44,200 parts per million. In view of this large quantity of TNT, it is somewhat surprising that the TNT transformation products are not present at greater concentrations. Sediment redox potentials measured here were quite high, despite an apparent buildup of reduced nitrogen forms. The likelyhood of 2,4,6-TNT transformation to products currently not under study appears great. These enigmas are discussed below.

ENVIRONMENTAL CHEMISTRY OF MUNITIONS COMPOUNDS

The extreme chemical reactivity of trinitrotoluene and other polynitro aromatics severly complicates the investigation of environmental fate of these compounds. Such high energy materials can react in a variety of ways, depending primarily on the presence of co-reactants and catalytic factors. In natural aquatic enivronments, the potential pathways for transformation of TNT are abundant. However, according to current theories, two major processes predominate: 1) photochemically mediated

transformation, involving a large number of oxidation and reduction reactions, and 2) non-photo energized reduction, which is frequently biologically mediated.

The photochemistry of aromatic nitro compounds has been studied in great detail[41]. Research on the photolysis of alpha TNT, in particular, has been conducted in increasing amounts during the last five years, as a direct result of concern over treatment of wastewater bearing residual nitrobodies. Large quantities of so-called "red water" and "pink water" can be generated daily at facilities where munitions compounds are synthesized. In addition, such wastewaters are generated in large quantities during wash-down procedures at munitions assembly installations, owing in part to the low solubility of most polynitro aromatics in water (e.g. alpha TNT is soluble to about 125 ppm in water at 25°C). In some cases, the most concentrated of these wastewaters are incinerated as a means of disposal. However, the incineration of up to 1,000,000 gallons per day of low concentration "pink water" is financially prohibitive. Consequently, such wastewaters are usually neutralized and discharged to surface streams and lakes. The adjustment of pH in acidic wastes bearing aromatic nitrobodies, and the photolytic exposure of these neutralized wastewaters results in the formation of intensely colored solutions containing a wide variety of transformation products.

The most commonly used nitrobody in military explosives is trinitrotoluene. Of the possible isomers of this material, 2,4,6-TNT (alpha TNT) is by far the most abundant in munitions formulations. Several researchers have been studying the photolytic activity of alpha TNT as a preliminary step in understanding the toxicity of its photolysis products and developing a means of treating wastewater containing them[11-12]. At a recent conference entitled "Symposium on Munitions Standards Research" and sponsored by the U. S. Army Medical Research and Development Command, 16 different photolysis products of 2,4,6-TNT were identified. A list of these is presented in Table 22. Many of these compounds are

Table 22. KNOWN PHOTOLYSIS PRODUCTS OF 2,4,6-TNT

1,3,5-Trinitrobenzene

1,3-Dinitrobenzene

2,4-Dinitrotoluene

2,4,6-Trinitrobenzaldehyde

2,4,6-Trinitrobenzyl Alcohol

2,4,6-Trinitrobenzonitrile

2,4,6-Trinitrobenzaldoxime

4,6-Dintro (1,2) Benzisoxazole

4,6-Dintroanthranil

3,5-Dinitrophenol

2,4,6-Trinitrobenzoic Acid

2-Amino-4,6-Dinitrobenzoic Acid

2,2'-Dicarboxy-3,3',5,5'-Tetranitro-Azoxybenzene

2,2'-Dicarboxy-3,3',5,5'-Tetranitro-Azobenzene

2-Carboxy-3,3',5,5'-Tetranitro-Azoxybenzene

N-(2-Carboxy-3,5-Dinitrophenyl)-2,4,6-Trinitrobenzamide

chemically unstable and doubtless are subject of further transformation. In laboratory studies where aqueous solutions of TNT were photolyzed, the compounds listed in Table 22 accounted for less thant 50 percent of the TNT which disappeared. The remaining fraction has yet defied identification by several research teams currently working on this problem [12]. Obviously, the full impact of discharging alpha TNT into the environment can never be understood until the major steps in its photolytic transformation are identified and the toxicity and persistance of intermediate and end products is tested.

This is much the case for the non-photo energized reduction of 2,4,6-TNT. Channon reported in 1943 that the metabolism of alpha TNT in rabbits produced two monoamine and one hydroxylamine transformation products [17]. He estimated that up to 30 percent of the administered TNT was metabolized to aromatic amine compounds, with most of the remaining transformation products being glucuronide complexes. In more recent work, Won, et. al.[2], found certain microorganisms could rapidly metabolize TNT to reduced species similar to those observed by Channon [4]. They also found, however, that these species were further reduced to diamino derivatives, which were resistant to further metabolism. In 1974, McCormick concluded that under anaerobic conditions alpha TNT could be converted microbiologically to triaminotoluene. Sitzmann[17] undertook the chemical reduction of 2,4,6-TNT and produced a variety of compounds, which are listed in Table 23. Many of these products are the same ones found in biological metabolism studies, indicating that reduction is a major mechanism in the transformation of 2,4,6-TNT in living organisms.

Other information is available on the reduction of aromatic nitro-compounds since they are widely used in the chemical industry as intermediates in synthesis. An important feature of these compounds is the variety of substances which can be obtained by their reduction. In general, reduction under acidic conditions produces amines, reduction under neutral conditions produces hydroxylamines, and reduction under basic

conditions produces dimers such as hydrazo, azo and azoxy compounds [18]. A generalized flow diagram for the reduction of nitrobenzene appears in Figure [10].

Appreciable evidence exists that some or all of the reduction processes described above are operating in the environment. This is particularly true in sediment systems where redox reactions can occur without massive influence from molecular oxygen. Nitrate has been found to poise sediments at an Eh of 100 to 200 mv. Depressions in Eh were not observed until nitrate reduction was complete [19]. It is not unlikely that the nitro groups in TNT also serve to poise the sediment redox condition. Reduction of the nitro groups should correlate with lower sediment Eh, although it is highly doubtful that the nitro groups in TNT participate in a measurable, reversible redox couple. The recorded Eh at JAAP station T1 varied between 250 and 400 mv. The nitro-bodies measured in these sediments were quite high; a moderate enrichment in Kjeldahl-nitrogen was observed. At station T2, the nitro-body content was much lower, while the Kjeldahl-nitrogen level rose very significantly. The Eh measured here ranged from 30 to 160 mv - well below the nitrate redox "boundary". Eh recorded in core samples taken from station T1A were high, hovering around 360 mv. The level of reduced nitrogen was noticeably high, through the concentration of alpha TNT was outstanding. This may mean that while TNT can serve as an electron acceptor, and thus help poise the redox condition, it does not participate in the couple being measured with the electrode system. If the role as an electron acceptor is correct, then the appearance of hydroxylamine, amine and other reduced transformation products is predictable. The fact that the hydroxylamine reduction products measured at JAAP stations T1A and T2 account for so little transformation of TNT suggest that other products must exist in this type of sediment where reduction is likely to occur.

FIGURE 10. FLOW DIAGRAM FOR THE REDUCTION OF NITROBENZENE[18]

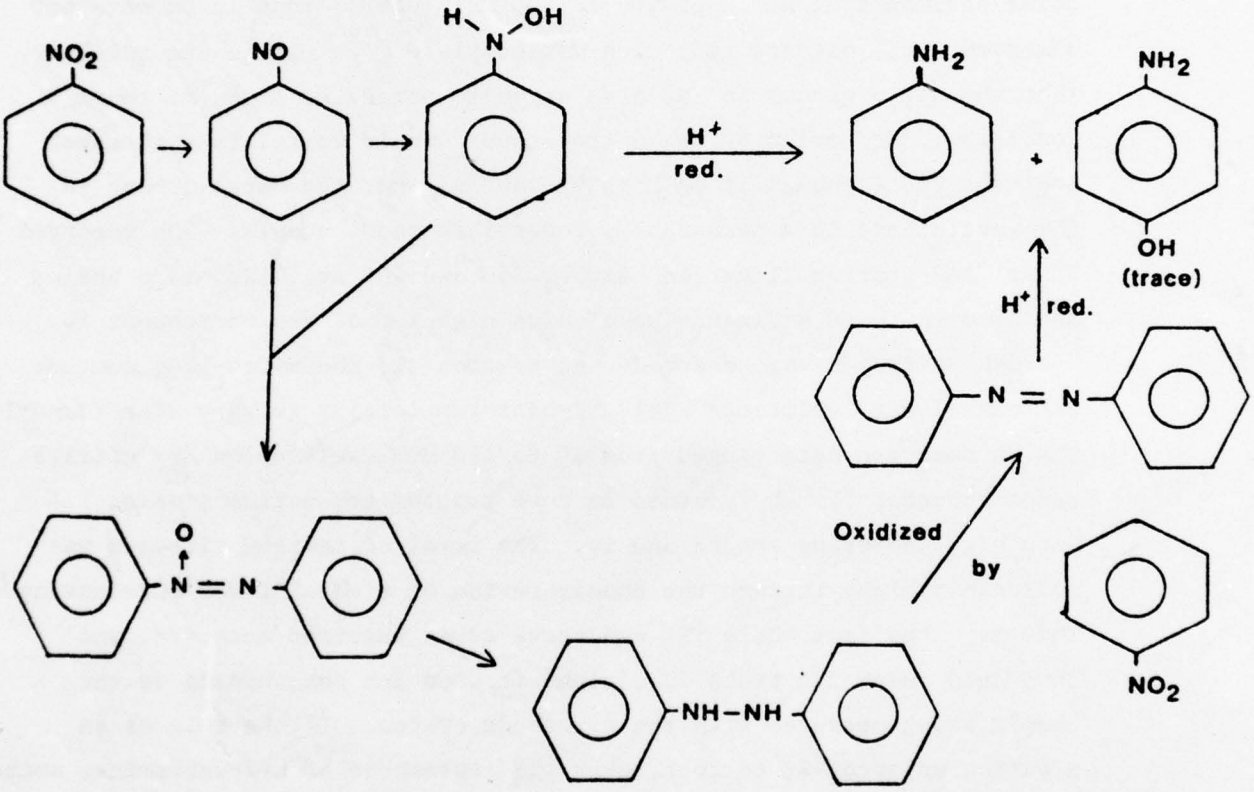


Table 23. CHEMICAL REDUCTION PRODUCTS OF 2,4,6-TNT FOUND BY SITZMANN [C-12]

4-Hydroxylamino-2,6-Dinitrotoluene

2-Hydroxylamino-4,6-Dinitrotoluene

4-Amino-2,6-Dinitrotoluene

2-Amino-4,6-Dinitrotoluene

2,4-Diamino-6-Nitrotoluene

2,2',6,6'-Tetranitro-4,4'-Azotoluene

2,2',6,6'-Tetranitro-4,4'-Azoxytoluene

4,4',6,6'-Tetranitro-2,2'-Azoxytoluene

2,4'-Dimethyl-3,3',5,5'-Tetranitro-ONN-Azoxybenzene

2,4'-Dimethyl-3,3',5,5'-Tetranitro-ONN-Azoxybenzene

2,2'-Diamino-6,6'-Dinitro-4,4'-Azoxytoluene

2-Amino-4-Hydroxylamino-6-Nitrotoluene

SECTION VI

MICROBIOLOGY

ANALYTICAL PROCEDURES

Bacterial Enumeration

Enumeration of planktonic bacterial populations was accomplished utilizing spread plate techniques[20]. Viable plate counts were performed on the original water sample by making 10X serial dilutions, adding 0.1 ml of the dilution to partially dried trypticase soy agar plates (reduced concentration) spread aseptically with a bent glass rod and allowed to incubate at room temperature.

Microbiological Inhibition Study

The microbiological inhibition testing procedure for monitoring the oxidation rate of a microbiological seed at various dilutions of stream water was performed according to the methodology as described by Marks[21].

A series of BOD bottles were prepared with an added microbiological culture seed maintaining an oxygen consumption rate of 1 mg/l/hr. Glucose was added to all bottles to insure the presence of biodegradable organic materials. The bottles were incubated at 20°C for a period of three to five days at which time the final dissolved oxygen measurements were made in all bottles.

Benthic Microbiological Activity

Sediment Dissolved Oxygen Uptake -

The upper 5 cm of microcores collected from various sampling stations were utilized to determine the effects of Army specific munition compounds on oxygen utilization of benthic bacterial populations.

Five grams (wet weight) of sediment were placed in BOD bottles which were brought to volume with oxygen saturated BOD dilution water[7]. The uptake of dissolved

oxygen by indigenous microbiological populations was monitored every 24 hours utilizing a selective membrane dissolved oxygen probe. Additional carbon sources and TNT were added to the sediment to determine stimulatory or inhibitory effects on dissolved oxygen utilization.

Dehydrogenase Activity -

A preliminary study of the metabolic activity of the microbial populations in sediment samples was undertaken utilizing measurement of the dehydrogenase activity. The reduction of 2, 3, 5 triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) has been used to estimate the dehydrogenase activity of soil microflora[22-25].

Two milligrams (wet weight) of sediment were placed in outgased centrifuge tubes. Three tubes were utilized per station: a) control with $HgCl_2$. added; b) a tube with increased substrate concentrations (sodium citrate); c) a tube with increased substrate concentrations and increased TNT concentrations (100 mg/1). The concentrations of reagents utilized were: 1) TTC solution - 1g of 2,3,5 triphenyl tetrazolium chloride in 100 ml of distilled water; 2) Tris buffer - 6.037g of tris aminomethane plus 2ml of 1N HCl in 100 ml distilled water, pH adjusted to 8.4; 3) sodium citrate solution - 7.4g sodium citrate in 100 ml distilled water; 4) TNT solution - 500 mg of TNT per liter of distilled water. Added to each tube were 3 ml of tris buffer, 5 ml of sodium citrate solution and 5 ml of TTC solution. Four milliters of the TNT solution were added to the third tube. The total volume was made up to 20 mls with outgassed distilled water. The tubes were allowed to incubate at room temperature in the dark for 48 hours.

Following the incubation period the microbial activity was terminated by the addition of 20 mls of methanol which also extracts TPF. The extraction procedure involved agitating the sample every 15 minutes over a 3 hour period. The mixture was then centrifuged and the absorbance of supernatant at 485 nm and 540 nm was determined by means of a spectrophotometer using methanol as a

blank. The difference in absorbance between the untreated and the $HgCl_2$. treated samples represents the dehydrogenase activity of the sediment.

Concentrations of TPF were calculated by comparison with a standard curve of TPF in methanol. Results were recorded as the amount of hydrogen transferred during the reduction of TTC to TPF in the sediment, according to the equation: 2,3,5 triphenyl chloride + 2H → triphenylformazan + HCl. The formation of 1 mg of TPF requires 150.35 ml H^{26} .

Sediment ATP Activity -

Microbiological activity in the sediment was determined utilizing the luciferin-luciferase bioluminescence technique for determining adenosine triphosphate concentrations. The luciferin-luciferase mixture used was obtained from Dupont Instrument Company. The Dupont 760 Luminescence Biometer was utilized to measure the luminescence of the reaction. The following is a simplified representation of the bioluminescence reaction[27] :

$$ATP + LH_2 + E \xrightarrow{Mg++} E\text{-}LH_2\text{-}AMP + P\text{-}P$$

$$E\text{-}LH_2\text{-}AMP + O_2 \longrightarrow E + \text{product} + CO_2 + AMP + \text{light}$$

ATP standards were prepared using adenosine 5' - triphosphate disodium salt trihydrate supplied by Dupont. The net light response versus ATP concentration exhibited a linear response.

Stationary phase cells of Eschericia coli K 12 were obtained by inoculating a nutrient broth solution and incubating for a 12 hour period to provide approximately 10^8 - 10^9 viable cells/ml. These cells were used on an internal standard in sediment extractions for determination of percent recovery. Chemical ATP standards were also used in some experiments.

Sediment samples were stored moist at 4^oC in sealed polyethylene containers. The maximum storage time prior to extraction was 60 days. ATP was extracted

according to the method of Bancroft[28]. Sixteen mls of boiling $NaHCO_3$ (pH 8.5) were added to 1cc of sediment and vortexed for one minute. The sample was centrifuged at 3,000 rpm for ten minutes. The supernatant was decanted and brought up to 30 ml volume with tris buffer. The assay for ATP was accomplished by making 1/6 and 1/10 dilutions of the supernatant and adding 10 µl of the diluted sample to 0.1 ml of the reaction mixture in the DuPont biometer.

ATP recovery from the sediment samples was determined by the addition of one ml of E. coli cells to one cc of sediment using the formula:

$$\% \text{ Recovery} = \frac{(\text{Sediment} + \text{Bacterial ATP}) - \text{Sediment ATP}}{\text{Bacterial ATP}} \times 100$$

IOWA

Results and Discussion

Microbiological Inhibition Study -

The microbiological inhibition study was performed with water samples collected at industrial outfalls I3, I4, I5, and I7. Effluent water was added to the BOD bottles at 0.0 ml, 10 ml, 100 ml and 250 ml concentrations. A five day incubation period was utilized. The final dissolved oxygen concentrations are shown in Table 24.

Concentrations of 2,4,6-TNT in the industrial outfalls on the sampling date ranged from <0.2 ppb at I3 and I5 to 8.4 ppb at I4. The transformation product, monohydroxylamino-dinitrotoluene was detected at 16 ppb levels in outfall I4.

These concentrations of TNT and the transformation product found in the industrial effluents did not retard the biological oxidation of organic compounds by the microbiological seed. Similar results were obtained in previous studies utilizing stream water collected from the Brush Creek[1].

Table 24. IOWA ARMY AMMUNITION PLANT - MICROBIOLOGICAL INHIBITION STUDY (Final D.O. mg/l Five Day Incubation)

Dilution*	I3	I4	I5	I7
1	> 0.2	> 0.2	> 0.2	0.6
2	> 0.2	> 0.2	0.3	0.6
3	> 0.2	> 0.2	> 0.2	0.3
4	0.3	0.6	> 0.2	> 0.2

Dilution

1- control

2- 10 ml sample

3- 100 ml sample

4- 250 ml sample

Studies have indicated that low concentrations of TNT retard BOD reduction and the self-purification of streams.[3,32,33,34] Enzinger[3] was able to adapt a bacterial species isolated from activated sludge to grow in a medium containing 100 ppm TNT although this concentration retarted the growth rate of non-adapted cultures. Pseudomonads isolated from sewage treatment plants have also been reported to degrade 100 ppm TNT [3].

Sediment Dissolved Oxygen Uptake -

The rate of consumption of dissolved oxygen from overlying waters is a rapid and sensitive index of benthic community metabolism. Table 25 shows the remaining dissolved oxygen following 24 and 96 hours incubation. Rates have been calculated for each station on the basis of sediment oxygen consumption and are as follows: B1 - 17.9 mg $O_2/m^2/hr$; B2 - 28.6 mg $O_2/m^2/hr$; B3 - 35.8 mg $O_2/m^2/hr$; and B4 - 28.6 mg $O_2/m^2/hr$. Sodium citrate as an added carbon source greatly increased the uptake rate as the dissolved oxygen was utilized within 24 hours.

These data indicate that the biological sediment activity is greatest at station B3. Stations B2 and B4 exhibited similar rates of oxygen uptake followed by station B1. Analysis of sediment total volatile solids and chemical oxygen demand data provide little insight into the oxygen uptake values. Previous studies have indicated that sediment oxygen uptake was not correlated with total organic matter in the sediment. However, as indicated in Table 25 , if the available carbon is easily utilized by the microbiological community a significant increase in dissolved oxygen uptake results.

Reported oxygen consumption rates of various sediment types ranged from 6 to 410 mg $O_2/m^2/hour$ with near saturated oxygen conditions in the overlying water [29].

Electron potential measurements were obtained from microcores to determine the redox conditions existing in the sediments. Under aerobic conditions

TABLE 25. IOWA AAP
BENTHIC MICROBIOLOGICAL ACTIVITY
(DISSOLVED OXYGEN -mg/1)

STATION	SAMPLE	24 hr.	96 hr.	mgO_2 consumed/m^2/hr
B1	1	7.2	7.0	
	2	5.9	2.6	17.9
	3	0.3	<0.2	103.8
B2	1	6.6	6.5	
	2	4.6	1.3	28.6
	3	<0.2	<0.2	≥ 96.7
B3	1	7.1	6.9	
	2	4.6	1.9	35.8
	3	<0.2	<0.2	≥ 103.8
B4	1	6.7	6.4	
	2	4.7	1.2	28.6
	3	0.4	<0.2	93.1

1 - control

2 - sediment only

3 - sediment with 340 mg/1 sodium citrate

AD-A036 778 ENVIRONMENTAL CONTROL TECHNOLOGY CORP ANN ARBOR MICH F/G 13/2
AQUATIC FIELD SURVEYS AT IOWA, RADFORD, AND JOLIET ARMY AMMUNIT--ETC(U)
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molecular oxygen serves as the terminal electron acceptor in microbial metabolism. When the dissolved oxygen is depleted alternate electron acceptors are utilized. Average values at 0-2 cm depth in Brush Creek sediments ranged from 300-400 mv which are indicative of aerobic and microaerophilic conditions. Station B3, however, eshibited Eh measurements around 0-10 mv at which sulfates are utilized as terminal electron acceptors. The results of these determinations indicate that the majority of these benthic communities are well aerated and most of the oxygen consumption in the sediments is the result of biological respiration.

Sediment ATP Activity -

The results of ATP determinations from upstream Brush Creek stations are shown in Table 26. Good reproducibility is shown with the recovery of ATP from the bacterial standards alone. Sediment determinations exhibited a fairly wide range of values but were within the same magnitude. Calculated percent recoveries were high and exceed 100 percent in two samples.

Stations B2 and B3 exhibited the highest ATP concentrations which may have been due to the ease of extractability from these sediment types. The sediments at B2 and B3 consisted primarily of coarse sand while those at B1, B4 and B5 contained clay and/or detrital material.

Previous studies have indicated that ATP extraction efficiencies vary significantly depending on the technique chosen[30] . Two factors which were routinely correlated with low extraction efficiencies were high moisture content and high clay content. The interactions of inorganic ions present in the sediment have also been alluded to as reasons for poor extraction efficienciencies in sediments [31] .

The extraction efficiency of ATP from bacterial standards was calculated prior to sediment extractions. Ranges of 3.0 to 6.0 µg/ ATP/mg cell dry weight have been reported for E. coli in various growth phases. Our experimental results indicated an average value of 2.1 µg ATP/mg cell dry weight for early stationary growth with a substantial variation

TABLE 26 . IOWA AAP
BENTHIC MICROBIOLOGICAL ACTIVITY
(ATP Concentration* - $\mu g \times 10^{-2}$
ATP/g sediment dry weight)

Station	Sediment'	Bacterial Standard	Sediment with Bacterial Std.	Percent Recovery
B1	4.33 ± 0.61	4.33 ± 1.81	7.97 ± 1.38	84
B2	8.28 ± 1.32	4.43 ± 1.01	13.69 ± 1.14	122
B3	6.00 ± 0.75	3.44 ± 0.12	12.02 ± 0.86	175
B4	2.30 ± 0.29	4.36 ± 0.97	6.19 ± 0.22	89
B5	2.19 ± 0.13	4.43 ± 1.01	5.50 ± 0.50	75

* mean ± std. dev.

' upper 0-2 cm core

which approached an order of magnitude.

Percent recoveries obtained exceeding 100 percent may be due to the time lapse from the extraction of the bacterial standard and addition and extraction of the internal standard in the sediment fraction. This lag period may have caused an increase in total cell number and biomass. Levels of cellular ATP have been shown to vary ten-fold in pure culture extractions [31], while other authors have shown no appreciable changes in ATP content v.s. growth stage [27].

Limited studies with sediment ATP values have reported figures ranging from 0.2 - 0.6 µg/kg sediment dry weight[31]. The values determined from Brush Creek sediments ranged from 0.02 - 0.08 µg/kg sediment dry weight which may be indicative of incomplete ATP recovery or reduced biological activity being present in these sediments. An average value of 0.5 femtogram (10^{-15} gram) ATP/cell has been reported for bacteria[27]. Utilizing this value to calculate total bacteria per gram sediment a figure of approximately 10^7 organisms is obtained. This value correlates with total counts done in the sediment at these stations in a previous study[31].

JOLIET

Results and Discussion

Bacterial Enumeration -

Semi-quantitative evaluation of the aerobic heterotrophic bacterial population in the stream at Joliet sampling stations are shown in Table 27.

Table 27. JOLIET AAP - PLANKTONIC BACTERIAL PLATE COUNTS

Station	Colonies/ml
G1	1.57×10^5
G2	1.47×10^5
T1	1.14×10^5
T2	165

The bacterial populations remained fairly consistant at the two stations sampled in Grant Creek and the upstream station in the TNT ditch. The reduction in the bacterial population observed at station T2 was probably due to the pH 2.7 observed at the time of sample collection.

The plate counts obtained, with the exception of station T2, were within the reported ranges for bacterial enumeration in stream water.

Semi-quantitative enumeration of the aerobic heterotrophic bacterial populations in the upper one cm of the sediment are shown in Table 28 .

Table 28. JOLIET AAP - SEDIMENT BACTERIAL PLATE COUNTS

Station	Colonies/ml
G1	7.92×10^7
G2	5.31×10^7
T1	5.21×10^7
T2	2.32×10^7

A large segment of the populations were Pseudomonas - like organisms qualitatively differentiated due to pigment production.

Filamentous fungi and yeasts were enumerated in aqueous samples from sampling stations at JAAP (Table29).

Table 29. FILAMENTOUS FUNGI AND YEASTS - JAAP

Station	Fungi	Yeasts
G1	540	200
G2	420	180
T1	280	40
T2	190	15

The majority of the fungal organisms were of the genera Penicillium, Fusarium, and Mucor. Observed yeast populations were low at stations T1 and T2. These samples were obtained prior to the slug flow of TNT

wastewater to avoid population inhibition due to low pH at station T2.

Microbiological Inhibition Study -

The microbiological inhibition study was performed with water samples collected at station G1 and G2 (Grant Creek), T1 and T2 (TNT ditch), and T1A, a small TNT disposal pond. Stream water was added to the BOD bottles at concentrations ranging from 0.0 ml to 250 ml. The results obtained following a three day incubation period are shown in Table 30.

The oxidation of organic matter by the added microbiological seed was not significantly retarded at the concentrations of stream water added from stations G1, G2, T2 and from the red water pond T1A. The inhibitory effects shown at the lowest dilution at station T2 was probably due to the low pH as the sample was not neutralized at this dilution. The low oxygen utilization observed at station T1A was probably due to the low O_2 uptake rate of the microbiological seed utilized for this sample.

Station G1 (Grant Creek) was utilized as the control station. Concentrations of TNT found at stations G2, T1, and T2 in the aqueous phase were 86 µg/1, 430 µg/1 and 1020 µg/1, respectively. Dinitrotoluenes and transformation products of TNT were also found at these stations. Concentrations of these are shown in Table 18.

These studies indicate that TNT concentrations encountered in the receiving stream at JAAP do not retard teh biochemical oxygen deamdn exerted by added organic compounds on an unacclimated microbiological seed.

Sediment ATP Activity -

The results of sediment ATP determinations from Joliet AAP are shown in Table 31. Station G1 exhibited the highest sediment activity based on ATP determinations while G2 exhibited the lowest activity. Stations T1, T2, and T1A had similar ATP values. The low activity found at station G2 may be due to the lack of ideal substrate conditions as this station consisted predominately of coarse sand. For a more complete discussion

TABLE 30. JOLIET AAP
MICROBIOLOGICAL INHIBITION STUDY
(Final D.O. (mg/l) - 72 hr. incubation)

Dilution*	G1	G2	T1	T2	T1A
1	1.9	2.5	2.7	2.5	4.5
2	1.2	1.9	3.5	3.6	5.0
3	2.0	0.7	3.1	3.6	4.9
4	2.5	2.7	3.5	0.9	4.6
5	2.8	2.3	1.7	1.5	6.3
6	2.4	2.1	<0.2	7.8	5.1

*Dilution

1-control

2-0.1 ml sample

3-1.0 ml sample

4-10 ml sample

5-100 ml sample

6-250 ml sample

TABLE 31. JOLIET AAP
BENTHIC MICROBIOLOGICAL ACTIVITY
(ATP Concentration*-µg x 10^{-2} ATP/
g sediment dry weight)

Station	Sediment'	Bacterial Standard	Sediment with Bacterial std.	Percent Recovery
G1	9.78 ± 1.38	4.63 ± 0.74	19.31 ± 1.83	205
G2	2.92 ± 0.20	4.45 ± 0.48	7.98 ± 1.86	114
T1	5.85 ± 0.23	9.07 ± 0.45	10.22 ± 2.36	48
T2	6.68 ± 0.44	7.80 ± 1.48	13.50 ± 0.80	87
T1A	5.91 ± 3.96	8.31 ± 0.17	17.05 ± 6.78	134

* mean ± std. dev.

' upper 0-2 cm core

of ATP values in sediments refer to IAAP section.

Sediment Dissolved Oxygen Uptake -

Microbiological activity at the Joliet AAP stations was determined by monitoring dissolved oxygen uptake which is a sensitive index of benthic community metabolism. Table 32 shows the dissolved oxygen remaining following sediment incubation, and dissolved oxygen uptake rates are shown in Table 33. Sample 2 represents the dissolved oxygen uptake normally occurring in the sediments of the selected stations. Station G1 exhibited the highest O_2 uptake, followed by G2 and T2. Oxygen uptake at stations T1 and T1A were similar. The effect of TNT added to the sediment samples was monitored as indicated by sample 4. Increased oxygen utilization was observed at stations G2 and T1. The increase at station T1 was approximately three fold. The addition of an excess of organic carbon (sodium citrate) showed a drastic increase in oxygen utilization (sample 3). The total dissolved oxygen was utilized within a 24 hour period in samples from stations G1, G2 and T1. A lag period was observed for stations T2 and T1A but the total available O_2 was utilized within a 48 hour period. The addition of TNT with the added carbon source did not have any effect on O_2 uptake (sample 5).

Sediment Dehydrogenase Activity -

The dehydrogenase activity of the sediment microcores were examined with added carbon sources and TNT as an indicator of toxicity of TNT to the indigenous microorganism. The microbial activity based on this enzymatic reduction with the JAAP samples is shown in Table 34.

A wide range of activities were observed at the various sampling stations. Stations with higher TNT concentrations exhibited greater dehydrogenase activity. Stations G1 (control) and G2 had similar values. Trinitrotoluene was not found in the sediment at G1 and concentrations of 1 mg/kg were found at station G2. The addition of TNT to these samples significantly increased the activity. Similar results were obtained with samples from stations T1 and T2, with the highest activity being observed at T1

TABLE 32. JOLIET AAP
BENTHIC MICROBIOLOGICAL ACTIVITY
(DISSOLVED OXYGEN mg/1)

STATION	SAMPLE	24 hr.	48 hr.	96 hr.	144 hr.	312 hr.
G1	1	7.1	7.1	6.9	-	-
	2	5.9	3.1	0.3	-	-
	3	0.3	-	-	-	-
	4	5.8	3.8	0.3	-	-
	5	0.3	-	-	-	-
G2	1	7.5	7.5	7.5	7.5	7.4
	2	6.8	5.9	4.1	1.2	0.3
	3	0.3	-	-	-	-
	4	6.6	6.1	5.2	3.2	1.7
	5	0.3	-	-	-	-
T1	1	7.5	7.5	7.6	7.7	-
	2	6.9	4.7	0.3	-	-
	3	0.3	-	-	-	-
	4	6.0	4.9	3.2	0.3	-
	5	0.3	-	-	-	-
T2	1	6.5	6.5	6.6	6.6	6.5
	2	5.8	5.0	4.0	1.8	0.3
	3	5.7	0.3	-	-	-
	4	5.7	5.1	4.3	2.0	0.8
	5	5.9	0.3	-	-	-
T1A	1	7.6	7.6	7.7	7.6	7.6
	2	7.2	6.9	6.0	5.8	5.2
	3	4.8	0.3			
	4	7.1	6.8	6.2	6.0	4.8
	5	5.2	0.3			

SAMPLE

1 control

2 sediment sample only

3 sediment with 340 mg/1 sodium citrate

4 sediment with 50 mg/1 TNT

5 sediment with 340 mg/1 sodium citrate and 50 mg/1 TNT

Table 33. JOLIET ARMY AMMUNITION PLANT
BENTHIC MICROBIOLOGICAL ACTIVITY
DISSOLVED OXYGEN UPTAKE RATES

Station	Sample	mgO_2 consumed/m^2/hr	
G1	1	-	
	2	17.9	
	3	100.2	
	4	17.9	
	5	100.2	
G2	1	-	
	2	10.7	
	3	107.4	
	4	14.3	
	5	107.4	
T1	1	-	
	2	7.1	
	3	107.4	
	4	21.5	
	5	107.4	
T2	1	-	
	2	10.7	
	3	10.7	93.1*
	4	10.7	
	5	7.1	93.1*
T1A	1	-	
	2	7.1	
	3	43.0	107.4*
	4	7.1	
	5	35.8	107.4*

*uptake rate following 48 hour incubation period

Sample
1. Control
2. Sediment sample only
3. Sediment with 340 mg/1 sodium citrate
4. Sediment with 50 mg/1 TNT
5. Sediment with 340 mg/1 sodium citrate and 50 mg/1 TNT

TABLE 34 . JOLIET AAP
BENTHIC MICROBIOLOGICAL ACTIVITY
Dehydrogenase Activity (per gram sediment dry weight)

Station*	Absorbance @ 540 nm	µl H transferred
G1-1	0.12	0.40
2	0.28	0.96
G2-1	0.14	0.44
2	0.28	0.96
T1-1	0.56	1.77
2	0.72	2.49
T2-1	0.20	0.72
2	0.32	1.04
T1A-1	1.56	9.24
2	1.20	5.6

* Sample

1 - 0.25% sodium citrate

2 - 0.25% sodium citrate + 100 mg/1 TNT

(338 mg/kg TNT). Sediment samples from station T1A had the highest dehydrogenase activity but showed a decrease in activity following the addition of 100 ppm TNT. This may be due to the initially higher amounts of TNT present in the sediment (44,200 mg/kg).

Reported values for dehydrogenase activity in sediments range from 0.2 - 100 μl H per gram of soil[26]. Our values were within this range although activity was low at select stations. Transformation products with spectral properties at the 540 nm band may have provided some interference in these analyses, although similar results were obtained at 485 nm.

SECTION VII

LABORATORY MICROCOSM STUDIES

INTRODUCTION

The objectives of these studies were: 1) to determine the persistance and/or degradation rates of the selected munition compounds under conditions optimal for aerobic and anaerobic decomposition, 2) to determine the major by-products of microbial degradation, and 3) to observe the toxicity of munition compounds on a diversified group of microorganisms.

Trinitrotoluene was added to microcosms optimized for aerobic and anaerobic metabolism in terms of microorganism diversity and metabolic versatility. The absence of observed degradation under such optimal conditions would strongly suggest that these compounds would persist in aquatic environments and thus be subject to concentration in sediments and higher forms of the food chain. Conversely, observed decomposition in these microcosms would suggest the need for determination of decomposition rates.

Observed disappearance of trinitrotoluene in the above microcosms does not constitute evidence of its complete mineralization to harmless inorganic products; alternatively this compound might be transformed to other derivatives that could be more toxic or detrimental to water quality. In the latter case the toxicities of the degradation products would become an important area of interest.

ANALYTICAL PROCEDURES

Microbiological Degradation Study

The upper 0.5 cm (1g wet weight) of sediment microcores collected from AAP sampling stations were suspended in 100 mls of sterile, buffered water and used as an inoculum for the microbiological degradation study.

Cultures were grown in 100 ml of a TNT enriched medium in 500 ml Klett flasks at 20°C on a reciprocating shaker at 85 excursions per minute. The culture medium was enriched to provide three different conditions: TNT as the sole carbon source, TNT with 0.01% nutrient enrichment, and TNT with 0.1% nutrient enrichment. The basal salt solutions consisted of: NaCl, $(NH_4)_2\ SO_4$, and $KH_2\ PO_4$ added aseptically to the medium of 50 ppm concentration of each from a stock solution of 10 g/l each with an adjustment to pH 7; and $CaCl_2$- 2.5 g/l, $CoCl_2 \cdot 6H_2O$ - 0.19 g/l, $FeCl2 \cdot 4H_2O$ - 0.2 g/l, $(NH_4)_6\ MoO_{24} \cdot 4H_2O$ - 0.1 g/l, $MgCl_2 \cdot 6H_2O$ - 2.5 g/l combined and added aseptically to the medium at 10 ppm concentrations. Glucose and acetate were added aseptically to the basal medium at 0.01% and 0.1% concentrations. Technical grade casamino acids were added to the basal medium at 0.1% concentrations. TNT was added to the medium at final concentrations of 10 mg/l and 100 mg/l, as determined by gas chromatography.

One ml of sediment inoculum was added to each of the flasks except the chemistry control. Culture growth was monitored for a 3-5 day growth period by a Klett colorimeter outfitted with a No. 42 blue filter (400-450 millimicrons). Absorbance values were determined by the equation:

$$\text{Absorbance} = \frac{\text{Klett units}}{500}$$

Samples for munitions compound analysis were prepared utilizing benzene extraction methods[1] . Twenty mls of reagent grade benzene was added to the culture flasks. The flasks were agitated and the benzene phase was extracted by means of a separatory funnel. Following three such extractions, the eluant was dried by passage over anhydrous sodium sulfate, concentrated to 5 ml and loaded on a 7 cm benzene wet-packed silica gel chromatography column. The column was eluted with 100 ml of 80:20 benzene/ethyl ether and the eluant was concentrated to dryness.

Thin layer chromatographic analyses were performed on activated and deactivated silica gel sheets with inorganic fluorescent indicator. Solvents used were benzene, benzene/ethyl ether (80:20), and benzene/toluene/hexane (10:10:5). Spots of the intermediates were visualized under ultraviolet illumination and by color reactions as described by Channon[4].

Analysis of the extracts by vapor phase chromatography involved utilization of SE 30 (10% w/w) solid phase coated on 80-100 mesh HP Chromosorb W support phase enclosed within a 6' x 14" O.D. column. A linear temperature programming rate of 15°C/min over a 100°-250°C temperature range was used.

High pressure liquid chromatography was also utilized for analysis of the extracts due to breakdown of some of the reported transformation products during vapor phase chromatography. Sample sizes of 100 µl were loaded onto a 1 ft. column packed with µ Porasil. The solvent systems used for partitioning and elution of the sample at 2000 psi were; chloroform/iso-octane (40:60) and chloroform/iso-octane/acentonitrile (15:84:1). Concentrations of the compounds eluted from the column were determined by absorbance values at 254 nm on a uv-visible spectrophotometer and by comparison with known standards.

Anaerobic Digesters

Laboratory-scale anaerobic digesters were employed to examine the effects of TNT on anaerobic metabolic activities and to observe the transformation of TNT under anoxic conditions as conditions optimum for methanogenesis were not observed at IAAP or JAAP as indicated by measured redox potentials of the samples. The digesters were slug fed daily with 25 mls of raw sludge obtained from a local wastewater treatment plant. A 500 ml gas collection cylinder was calibrated for measuring gas production. All digesters were kept in a constant temperature room at 35°C. As anaerobic conditions were not encountered in surface sediments at IAAP and JAAP, these studies were done to provide insight into the threshold toxicity levels at which acute effects on microbiological activity can be expected under anaerobic conditions.

The units were started from digested sludge obtained from local wastewater treatment plants. One month of operation was allowed prior to initiation of the studies to insure the establishement and equilibration of the bacterial population. All digesters were maintained at 400 ml sludge volume with a 16 day retention time.

Every 24 hours, gas volume and pH readings were taken, a 25 ml sample was withdrawn, and 25 mls of raw sludge was added. The digesters were well shaken

prior to withdrawal of the sample to insure sample uniformity, and were again shaken following the sludge addition.

Four digesters were utilized: 1) control; 2) solvent control; 3) low quantitites of TNT; and 4) high quantitites of TNT. Following acclimation of the digesters, large quantitites of TNT were added in crystalline form. Low concentrations of TNT dissolved in hexane/ethyl acetate (3:1) were added daily with the feed to account for TNT removal with sludge withdrawal.

Daily sludge withdrawals were solvent extracted and analyzed by vapor pressure and high pressure liquid chromatography for the parent compound and transformation products following procedures previously described. The extraction procedure involved acidifying the sludge with three volumes of distilled water adjusted to pH2 with 6N H_2SO_4. Twenty mls of reagent grade benzene were added to the samples, which were then agitated and the benzene phase was extracted by means of a separatory funnel. The unextracted volume (water phase) was adjusted to pH 12 with NaOH and triple extracted with benzene. The extraction procedure for the total digester volume (400 mls) involved drying, homogenization, and a four hour benzene solvent soxhlet extraction.

Lake Sediment Microcosm

Five cores of approximately 50 ml of lake sediment (upper 5 cm) obtained from a local, stratified lake were incubated at in situ temperatures (9°C) under anaerobic conditions for 2-3 weeks to measure the rate of methane production. Total gas production was measured manometrically and gas composition was measured by gas chromatography (Porapak Q, helium carrier). Methane production was computed from the gas analysis results and the total volume of each Warburg flask.

The five conditions established to determine the effects of TNT on gas production in lake sediments were: 1) control; 2) solvent control (10 µl cyclohexane); 3) TNT addition (10 mg/l TNT in 10 µl solvent); 4) solvent control (100 µl cyclohexane); 5) TNT addition (100 mg/l TNT in 100 µl solvent).

Gravel Percolater Microcosm

To delineate the effects of TNT on the metabolism of diversified microbiological populations, a gravel percolation microcosm was established.

The microcosm consisted of a 50 cm x 3 cm Pyrex column filled with pebbles (2-3 mm diameter) to a height of 15 cm. The basal salts medium as previously described with glucose (20 mg/1) and acetate (500 mg/1) added was sterilized and held aseptically in 5 liter volumes. The medium was percolated over the gravel at a constant rate of 1 ml/min. using a dekastaltic pump. The column was inoculated with biomass scrapings from trickling filter rocks which represented a variety of microorganisms and metabolic activities. Four percolaters were monitored, two controls and two with TNT added.

Following an acclimation period for population development, acetate utilization was monitored by gas chromatography. Influent and effluent samples were collected and prepared for analysis by adding 0.15 ml of phosphoric acid solution (20% meta-phosphoric acid, 1% ortho-phosphoric acid) per ml of sample followed by centrifugation. Ten microliters of the sample were injected onto a 6' x 1/4" O.D. column using FFAP as the solid phase coated on Gas Chrom A - 70/80 mesh. The column temperature was maintained at 135°C. Flame ionization was the type of detection utilized.

Disc Sensitivity Study

A filter paper disc method was utilized to evaluate the toxicity of munition compounds and related transformation products on a diversified group of microorganisms. Solutions of 50 mg/ml of the compounds were prepared in ethyl acetate or benzene. Twenty µl (1 mg of the compound) were loaded onto the discs which were allowed to air dry to facilitate solvent evaporation.

Trypticase soy agar was used as the growth medium. Agar plates were streaked with bacterial suspensions and the discs were placed on the inoculated plates. The plates were incubated for 48 hours at 35°C and analyzed by observing zones of growth inhibition in proximity to the discs.

RESULTS AND DISCUSSION

Microbiological Degradation Study

These studies were designed to observe the effects of TNT on enriched heterotrophic bacterial populations isolated from AAP sampling stations. Attempts were made to discern threshold toxicity levels and relative rates of TNT transformation in streams receiving low TNT waste discharges (IAAP) and those receiving high TNT waste discharges (JAAP). This study suggested that if a segment of the microbial populations in receiving streams accounts for the majority of TNT utilization, this fraction should be enriched for, and should contribute a large portion of the activity with respect to the utilization of TNT and the production of transformation products.

Sediment microcores collected on the fall survey from IAAP were utilized in this study. These cores were subjected to 10 ppm and 100 ppm concentrations of TNT and the results of the growth studies are shown in Table 35. The indigenous population was unable to utilize TNT as the sole carbon and energy source (cultures A and B). These organisms grown in the presence of additional carbon sources accelerated the transformation of TNT. This is a prime example of the theory of co-metabolism[39]. The growth curves are plotted for cultures C and D with the only variable being the amount of TNT (Figure 11). The biological controls for each station exhibited growth rates similar to culture C. These data show a similarity in growth rates but the total biomass is greater at higher TNT concentrations, possibly implying that TNT may be utilized as a carbon and/or energy source in the presence of an easily assimilated carbon source. The rates of TNT transformation were similar with populations isolated from the control station and populations receiving TNT wastes. The mean sediment muntions data for these stations are shown in Table 15.

Previous degradation studies with IAAP sediment populations showed that a high percentage of the transformation products were the monohydroxylamine-DNT[1]. In this study 10 ppm concentrations of TNT were approximately 90 percent transformed following three days incubation (Table 36). One isolated transformation product (D) was shown to occur in large quantities.

Table 35. IOWA AAP
EFFECT OF TNT ON THE GROWTH OF ENRICHED
INDIGENOUS BACTERIAL POPULATIONS (ABSORBANCE DATA)

	DAYS			
Culture*	0	1	2	3
B1A	.031	.030	.042	.038
B1B	.054	.068	.070	.066
B1C	.073	.418	.496	.504
B1D	.062	.322	.650	.696
Bio	.059	.398	.472	.496
B2A	.011	.012	.020	.016
B2B	.039	.036	.040	.040
B2C	.048	.308	.482	.424
B2D	.052	.182	.640	.702
Bio	.039	.304	.468	.437
B3A	.026	.040	.032	.036
B3B	.059	.064	.072	.064
B3C	.072	.430	.424	.386
B3D	.063	.350	.612	.706
Bio	.068	.421	.437	.411
B4A	.003	.002	.004	.008
B4B	.024	.026	.032	.036
B4C	.066	.118	.476	.454
B4D	.068	.106	.712	.692
Bio	.059	.124	.458	.432
B5A	.007	.008	.008	.014
B5B	.029	.032	.038	.032
B5C	.063	.356	.490	.476
B5D	.059	.266	.622	.664
Bio	.046	.337	.466	.458

*Culture Identification

A - 10 mg/1 TNT
B - 100 mg/1 TNT
C - 10 mg/1 TNT and 1.01% nutrients
D - 100 mg/1 TNT and 0.01% nutrients
Bio - Biological control, 0.01% nutrients

Figure 11
Iowa AAP - Growth Curves of Indigenous Microbial Populations
in Enriched Media

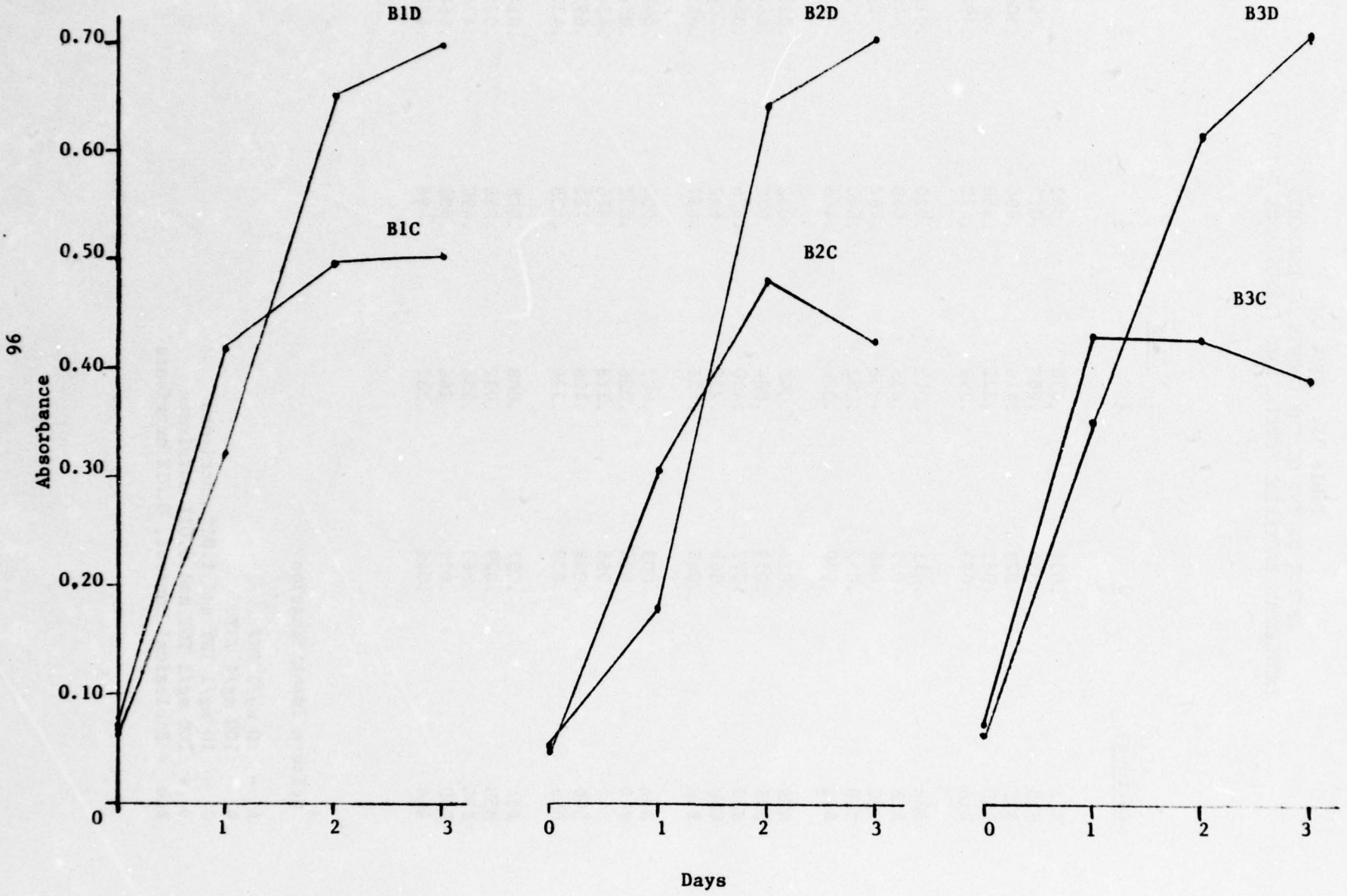


Figure 11(continued).

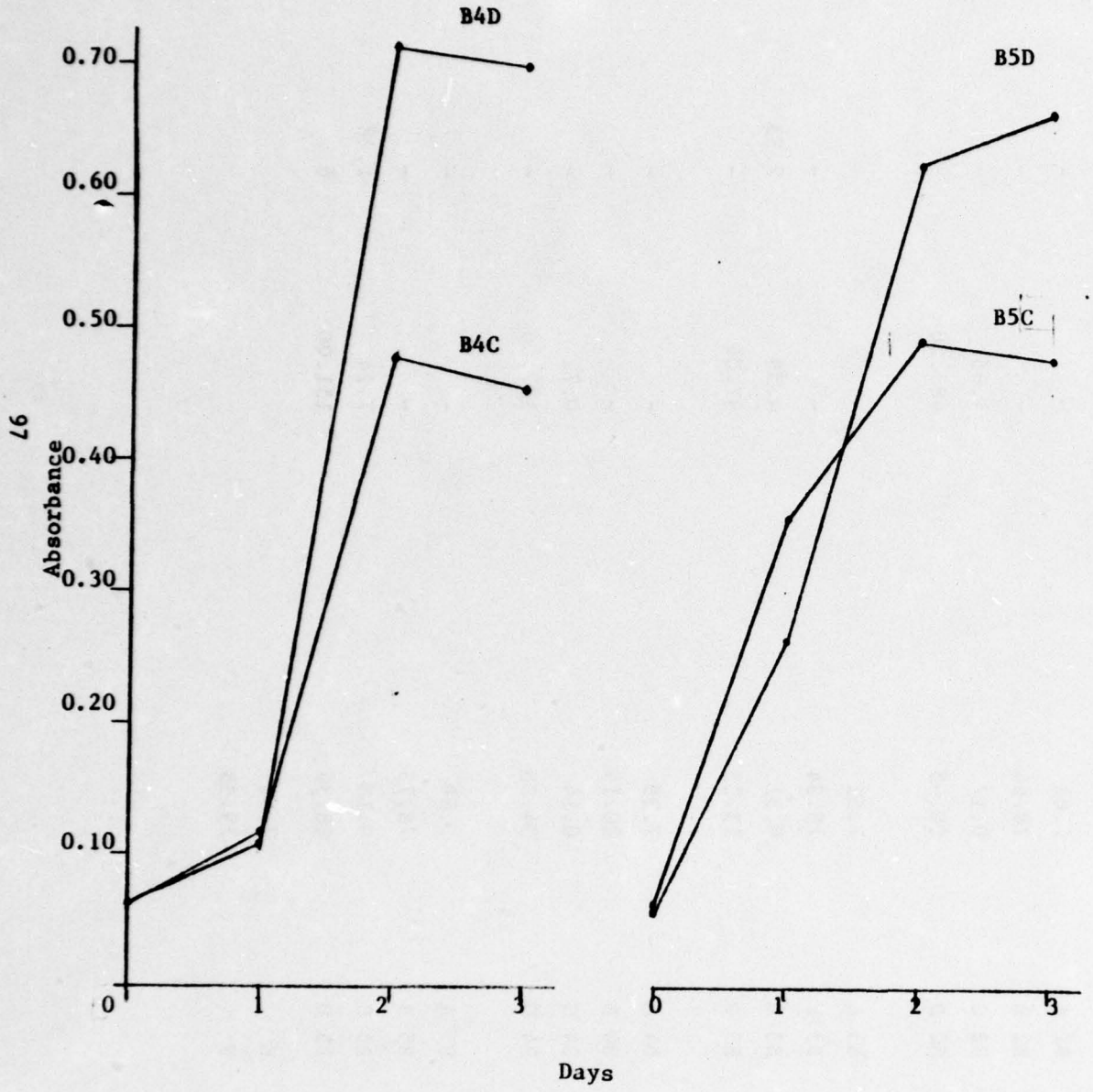


Table 36.
IOWA ARMY AMMUNITION PLANT
TNT UTILIZATION BY ENRICHED
INDIGENOUS BACTERIAL POPULATION

Station*	TNT Remaining' (mg/l)	Transformation Products' (relative area - mm^2)	
		D	E
B1 A	7.13	-	-
B1 B	82.11	-	-
B1 C	0.07	7.61	0.6
B1 D	29.08	188.38	-
B2 A	7.62	-	-
B2 B	78.64	-	-
B2 C	0.17	4.65	-
B2 D	30.45	485.30	-
B3 A	7.51	-	-
B3 B	76.34	-	-
B3 C	0.31	4.59	2.35
B3 D	13.33	97.06	-
B4 A	7.39	-	-
B4 B	80.14	-	-
B4 C	0.14	0.71	-
B4 D	34.26	187.06	-
B5 A	7.69	-	-
B5 B	78.75	-	-
B5 C	0.18	7.24	1.88
B5 D	28.38	151.06	0
E	7.83		
F	79.35		

Table 36. Continued

*Culture

A - 10 mg/l TNT, basal salt sol'n.

B - 100 mg/l TNT, basal salt sol'n.

C - 10 mg/l TNT, basal salt sol'n 0.01% nutrients

D - 100 mg/l TNT, basal salt sol'n 0.01% nutrients

E - Control, 10 mg/l TNT, basal salt sol'n, 0.01% nutrients

F - Control, 100 mg/l TNT, basal salt sol'n, 0.01% nutrients

' Reported values are the mean of two determinations following 3 day growth period

[1]Compounds identified as A, B, and C in Joliet Army Ammunition Plant studies were absent in this study.

This product was tentatively identified as the monoamine-DNT and accounted for approximately 4-10 percent of the TNT transformations products. Thin layer chromatograms of the benzene extracted cultures revealed similar compounds as shown in the previous report [1]. Cultures subjected to higher concentrations of TNT (100 ppm) exhibited a lower percent transformation of the parent compound (50-80%) but a correspondingly higher amount of transformation product D. Growth rates were not inhibited.

Bacterial populations isolated from JAAP sediments collected on the June survey 1975 were grown under similar conditions with 10 ppm and 100 ppm TNT concentrations. The growth of the these mixed populations is shown in Table 37 and Figure 12. These growth rates and total biomass values exhibit similar responses as shown with the Iowa cultures. Comparison of cultures grown under identical conditions with varying amounts of TNT showed an increase in total biomass values in the presence of 100 ppm TNT versus 10 ppm TNT (Figure 12). The rates of TNT transformation were similar to those obtained with Iowa cultures (Table 38 and 39). The majority of the cultures grown with 10 ppm TNT revealed greater than 90% transformation of the parent compound. The monoamine - DNT products (D and E) accounted for 6-10% of the transformation products formed. Cultures grown with higher concentrations of TNT (100 ppm) exhibited 50-80% transformation of TNT following a 72 hr. incubation period. The production of the "monoamine" compound accounted for 4-10% of the total transformation products. No inhibition of growth was observed with TNT, however high concentrations of nutrients coupled with high TNT concentrations seemed to retard growth.

Transformation studies with sample TIA exhibited variation in the amount of TNT utilized. TNT was also found in large quantities in the control. Chemistry data indicated the presence of 44,200 mg/kg TNT in the sediment (Table 21), which would account for the increase in TNT found in these samples. Two transformation products (A and B) appeared in the extracts of these cultures which were not found in the other culture extracts. These compounds may have been present in the sediment inoculum or may be

TABLE 37. JOLIET AAP
EFFECT OF TNT ON THE GROWTH OF
ENRICHED INDIGENOUS BACTERIAL
POPULATIONS (ABSORBANCE DATA)

	DAYS					
Culture*	0	1	2	3	4	5
G1A	.014	.106	.316	.312	.298	-
G1B	.033	.056	.436	.464	.472	-
G1C	.051	.074	.876	.990	.974	-
G1D	.093	.144	.144	.266	.924	1.13
G1E	.042	.048	1.014	1.35	1.39	-
G1F	.087	.356	.356	.378	.376	-
BIOA	.078	.114	.900	.982	.963	-
BIOB	.093	.242	.250	.760	1.14	1.26
G2A	.002	.004	.330	.386	.390	-
G2B	.015	.012	.446	.552	.598	-
G2C	.008	.010	.756	.950	.960	-
G2D	.094	.148	.150	.538	1.06	1.28
G2E	.013	.014	.784	1.23	1.31	1.32
G2F	.099	.328	.334	.336	.350	-
BIOA	.033	.046	.540	.950	.960	.982
BIOB	.102	.196	.196	.488	.900	.950
T1A	.051	.06	.304	.284	.290	-
T1B	.032	.054	.396	.458	.480	-
T1C	.044	.056	.944	.998	.984	-
T1D	.076	.092	.150	.162	.232	0.800
T1E	.042	.05	1.08	1.34	1.35	-
T1F	.098	.372	.360	.372	.380	-
BIOA	.063	.082	.884	.992	.990	-
BIOB	.082	.260	.254	.280	.260	0.630
T2A	.082	.114	.240	.240	.256	-
T2B	.069	.084	.374	.410	.460	-
T2C	.032	.038	.810	.860	.840	-
T2D	.098	.116	.132	.860	1.25	1.31
T2E	.073	.088	.976	1.104	1.16	-
T2F	.113	.364	.362	.370	.370	.374
BIOA	.054	.07	.800	.850	.860	-
BIOB	.124	.300	.288	.910	1.34	1.32

TABLE 37. Continued

Culture*	DAYS 0	1	2	3	4	5
T1AA	.148	.364	.378	.444	.470	-
T1AB	.172	.298	.430	.448	.480	-
T1AC	.163	.214	.896	.998	.994	-
T1AD	.187	.370	.364	.388	.400	-
T1AE	.159	.240	1.076	1.21	1.31	-
T1AF	.137	.580	.580	.604	.620	-
BIOA	.151	.210	.930	.946	.973	-
BIOB	.162	.498	.488	.500	.540	-

*Culture Identification

A	-	10 mg/l TNT, amino acids, basal salt solution
B	-	100 mg/l TNT, amino acids, basal salt solution
C	-	10 mg/l TNT, amino acids, 0.01% nutrients, basal salt solution
D	-	10 mg/l TNT, amino acids, 0.1% nutrients, basal salt solution
E	-	100 mg/l TNT, amino acids, 0.01% nutrients, basal salt solution
F	-	100 mg/l TNT, amino acids, 0.1% nutrients, basal salt solution
BIOA	-	0.01% nutrients without TNT
BIOB	-	0.1% nutrients without TNT

Figure 12
Joliet Army Ammunition Plant - Growth Curves of Indigenous Microbial Populations in Enriched Media

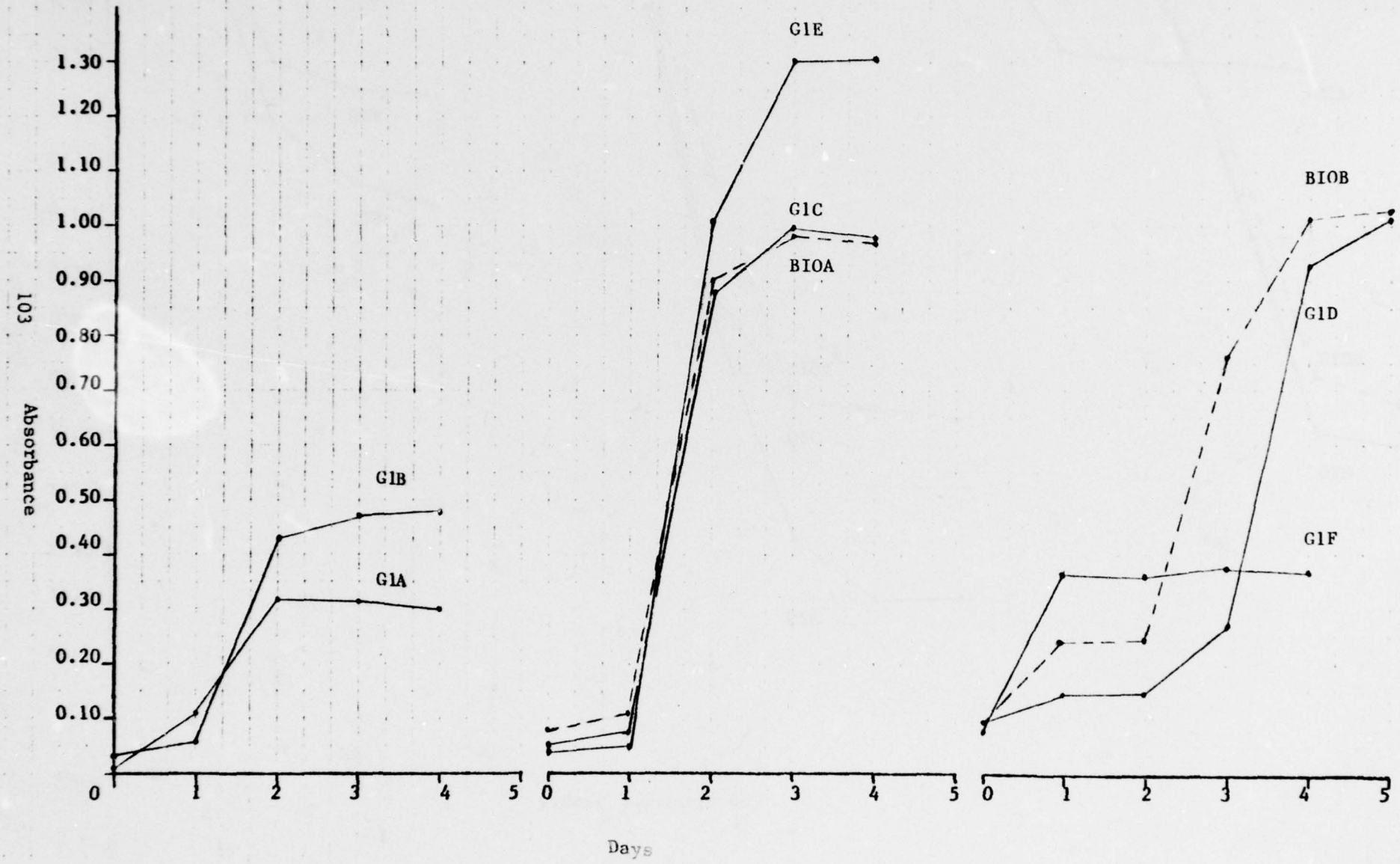


Figure 12(continued).

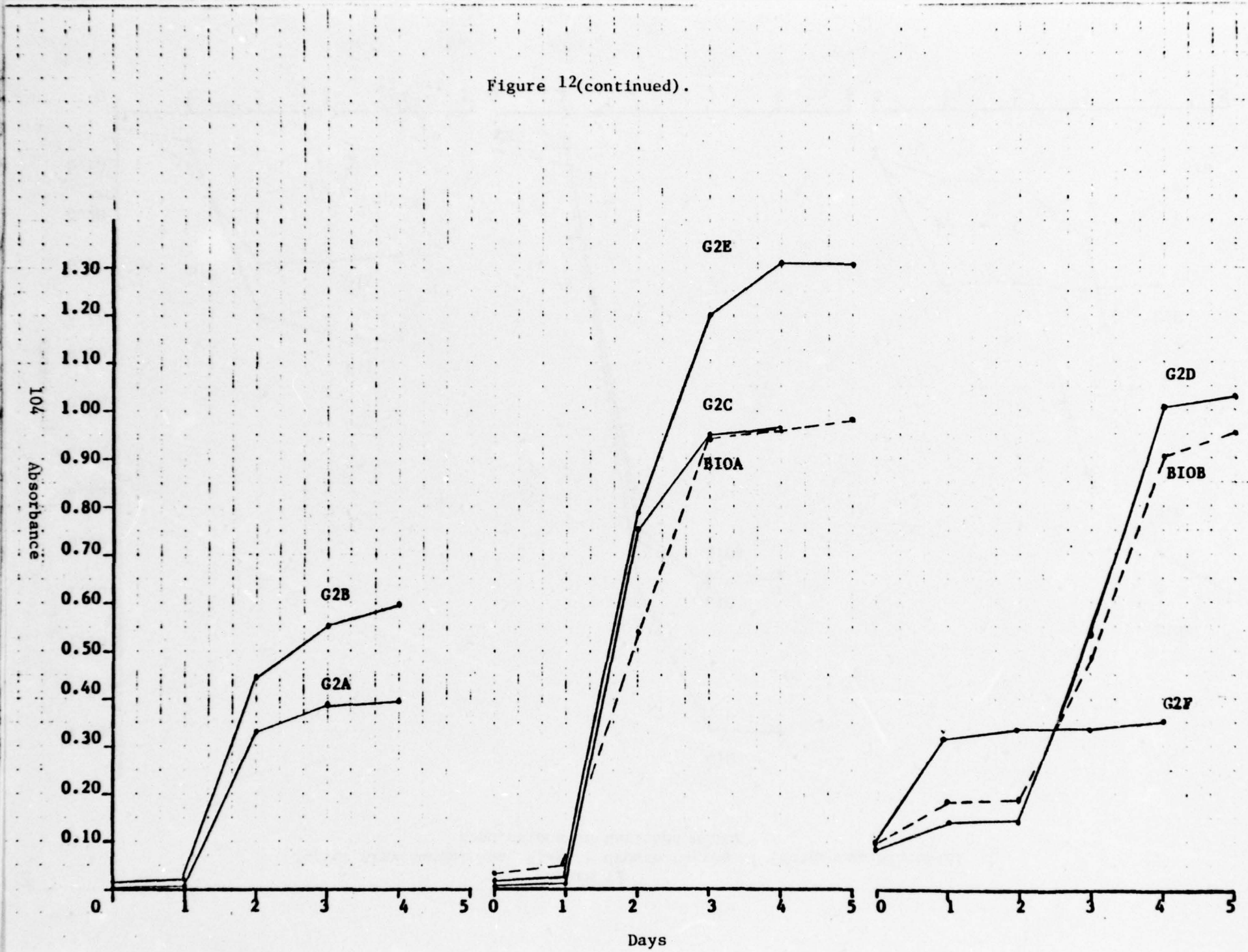


Figure 12 (continued).

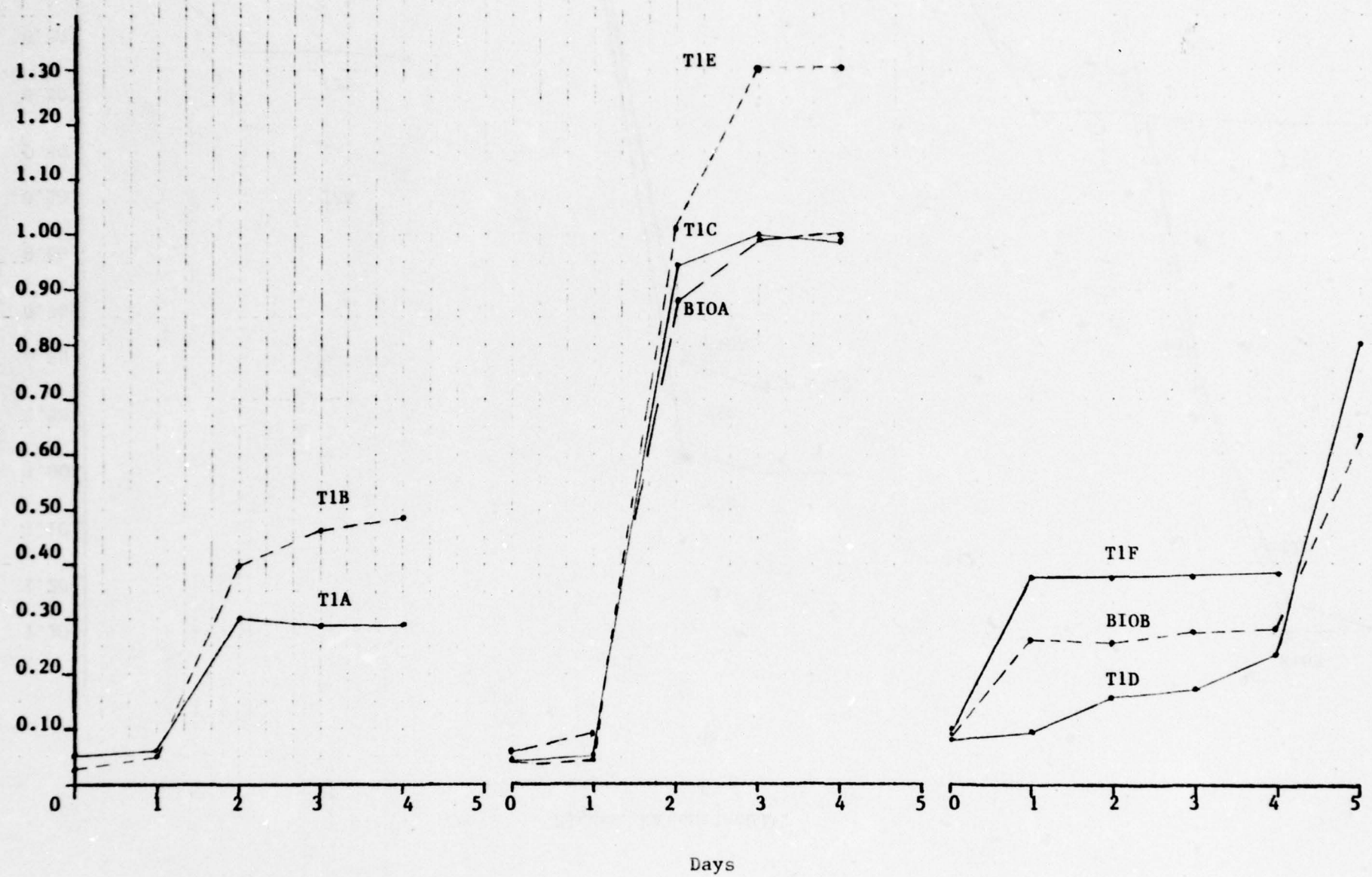


Figure 12(continued).

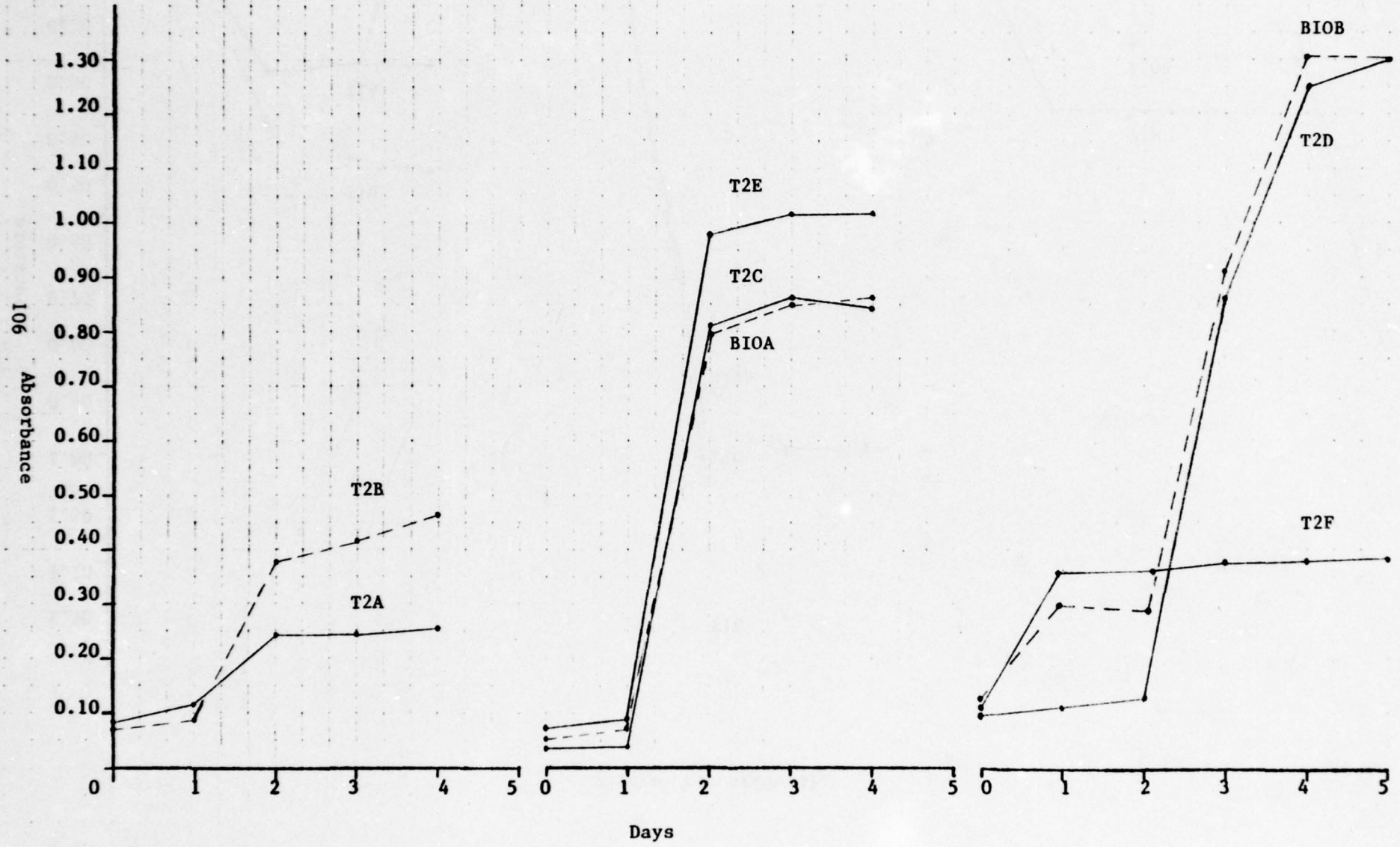


Figure 12 (continued).

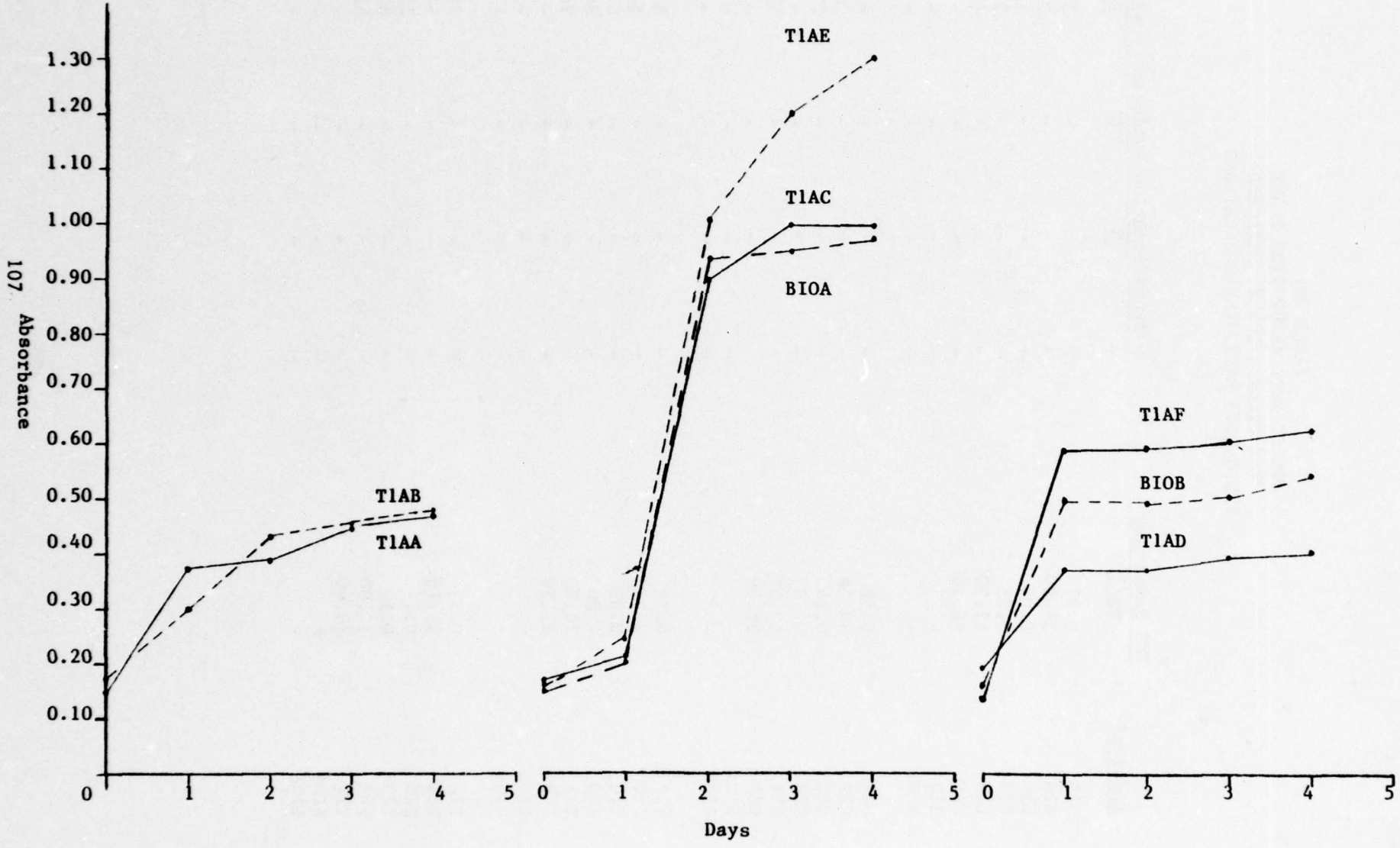


TABLE 38.
JOLIET ARMY AMMUNITION PLANT
TNT UTILIZATION BY ENRICHED
INDIGENOUS BACTERIAL POPULATIONS

Station*	TNT Remaining' mg/l	Transformation Products' (relative area mm^2) A	B	C	D	E
G1A	0.97	-	-	-	39	58
G1B	34.90	-	-	-	521	190
G1C	0.09	-	-	-	17	63
G1D	0.25	-	-	-	27	106
G1E	12.30	-	-	-	634	512
G1F	86.10	-	-	-	-	-
BIOA	-	-	-	-	-	-
BIOB	-	-	-	-	-	-
C2A	0.58	-	-	-	45	137
G2B	39.10	-	-	-	483	184
G2C	0.14	-	-	-	29	98
G2D	0.10	-	-	-	-	-
G2E	13.55	-	-	-	536	440
G2F	86.00	-	-	-	-	-
BIOA	-	-	-	-	-	-
BIOB	-	-	-	-	-	-
T1A	0.36	-	-	-	57	65
T1B	46.35	-	-	-	500	194
T1C	0.32	-	-	-	27	16
T1D	4.80	-	-	-	84	65
T1E	20.05	-	-	-	964	496
T1F	82.50	-	-	-	-	-
BIOA	-	-	-	-	-	-
BIOB	-	-	-	-	-	-
T2A	0.59	165	-	-	75	80
T2B	49.10	-	-	-	567	276
T2C	0.11	-	-	-	13	15
T2D	0.16	-	-	-	47	53
T2E	12.00	-	-	-	528	306
T2F	80.40	-	-	-	-	-
BIOA	-	-	-	-	-	-
BIOB	-	-	-	-	-	-

Table 38. Continued

Station*	TNT Remaining' mg/l	Transformation Products' (relative area mm^2) A	B	C	D	E
T1A A	17.80	357	1925	90	117	14
T1A B	85.70	692	2967	110	247	82
T1A C	5.45	432	1826	55	350	162
T1A D	51.25	389	1707	-	-	-
T1A E	69.60	564	2526	45	681	262
T1A F	138.00	1214	3941	-	-	-
BIO A	31.6	221	1958	32	526	316
BIO B	39.70	874	3544	-	-	-
Chem A	10.00					
Chem B	97.00					

*Culture

A - 10 mg/l TNT, basal salt sol'n, amino acids
B - 100 mg/l TNT, basal salt sol'n, amino acids
C - 10 mg/l TNT, basal salts, 0.01% nutrients
D - 10 mg/l TNT, basal salts, 0.1% nutrients
E - 100 mg/l TNT, basal salts, 0.01% nutrients
F - 100 mg/l TNT, basal salts, 0.1% nutrients
BIOA- biological control without TNT 0.01% nutrients
BIOB- biological control without TNT 0.1% nutrients
CHEM A- chemical control
CHEM B- chemical control

' reported values are the mean of two determinations.

Table 39.
JOLIET ARMY AMMUNITION PLANT
MICROBIOLOGICAL DEGRADATION OF TNT AND RESULTANT
PRODUCT FORMATION BY BENTHIC MICROBIAL POPULATIONS

Station*	TNT Remaining mg/l	Transformation Products (relative areas mm^2) A	B	C	D	E
G1-A	0	-	-	-	-	-
G1-B	5.46	-	-	-	56.88	20.00
G1-C	4.55	-	-	-	-	-
G2-A	0.02	-	-	-	40.00	15.00
G2-B	10.27	-	-	-	-	-
G2-C	8.82	-	-	-	127.5	63.75
T1-A	0.57	-	-	-	191.25	90.00
T1-B	11.36	-	-	-	80.00	25.00
T1-C	11.14	22.50	-	-	195.00	45.00
T2-A	0.01	-	-	-	-	-
T2-B	10.82	-	-	-	55.00	-
T2-C	1.69	-	-	-	660.00	980.00
T1A-A	29.46	640.00	2700.00	-	-	-
T1A-B	51.27	5040.00	16800.00	-	-	-
T1A-C	53.09	760.00	2600.00	-	-	-

* Culture Identification

A - Sediment only

B - Sediment, 100 mg/l TNT

C - Sediment, 100 mg/l TNT, 0.25% sodium citrate

products resulting from much higher TNT concentrations, and are suspected of being "azoxy" compounds.

High pressure liquid chromatography (HPLC) was employed in attempts at isolating and identifying TNT transformation products. In the isolation procedure the extracts were combined and concentrated. The concentrate was injected into the instrument using chloroform/iso-octane/acetonitrile (15:84:1) as the solvent system and the peak fractions were collected separately with the eluant. The chloroform/iso-octane/acetonitrile (15:84:1) solvent system was used for peak collecting due to its greater resolving power as compared with the chloroform/iso-octane (40:60) solvent system which was superior for rapid quantification. The isolated fractions were concentrated to approximately 2 ml. and reinjected to ascertain their purity. Attempts at identifying the isolates were accomplished through comparisons with standards run on gas chromatography, HPLC, and thin layer chromatography (Table 40). Compound A was suspected of being an "azoxy" compound due to similar Rf values and color reactions previously reported [4]. Compound B had unique retention times when compared to our standards but this was also suspected of being an "azoxy" compound. A weak infrared spectrum of this fraction showed evidence of an aromatic ring and nitro-groups suggesting a munitions origin. Compounds D and E had retention times similar to standards 2-amino-4,6-DNT and 4, amino- 2,6 DNT, respectively, when run on HPLC, GC and TLC. A weak infrared spectrum run of isolate D suggested that this compound may be an amide derivative.

"Hydroxylamine" - DNT standards exhibited much longer retention times with the HPLC system as compared to the "monoamine" standards. Extracts of selected samples thought to contain the "hydroxylamine" product were initially run at these longer retention times to determine the presence or absence of this compound. The presence of this compound was not detected in these samples and was therefore excluded from analyses due to long retention times required for detection.

Laboratory studies with indigenous sediment microbial populations were done to determine prevalent transformation and/or degradation products and to correlate these findings with environmental studies.

Table 40. AEROBIC DEGRADATION STUDY OF TNT USING MICROBIAL POPULATIONS FROM JOLIET ARMY AMMUNITION PLANT MAJOR TRANSFORMATION PRODUCTS VERSUS STANDARDS

Compounds	Solvent 60:40 iso-octane/chloroform	Solvent 84-15-1 chloroform/iso-octane/ acetonitrile	Gas Chromatography:seconds for system conditions see Figure 4 .	Thin layer Chromatography: R_f Solvent: 80:20 benzene/ethyl acetate Adsorbent:Activated Silica Gel	Developed Color of TLC Spots(2)
2,4,6-trintrotoluene	5	16	512	0.57	Brownish-Red
2,2',6,6'tetranitro-4,4'azoxytoluene	5	29	*	0.59	Medium Blue
Compound A	20	82	*	0.59	Pinkish-purple
Compound B(1)	33	138	1167	0.61	Orange
2 amino 4,6 dinitrotoluene	88	181	791	0.28	None
Compound D	89	178	777	0.26	None
4 amino 2,6 dinitrotoluene	99	205	753	0.33	None
Compound E	101	201	744	0.32	None

*Assumed to decompose (not demonstrated)

(1)No similar compounds could be found for standards

(2)Color is developed by spraying TLC plates with a 1:1:1 solution of methyl ethyl ketone/cyclohexnone/ 10% KOH.

The intensive survey determining microbial activity at Iowa AAP and Joliet AAP coupled with the laboratory studies were performed to determine threshold toxicity levels of TNT on microbiological communities existing in streams receiving munition wastes.

Contradictory literature exists on the effects of TNT on microbiological systems and the biodegradability of TNT. Early studies have indicate that TNT concentrations as low as 1 ppm in receiving waters affected the self-purification rates of streams [32]. Other studies have shown that TNT is less toxic and is degraded at much higher concentrations. Activated sludge units receiving 60 ppm TNT were not affected in waste treatability capabilities[33]. The biological treatability of TNT manufacturing wastewater was studied by Nay[34]. Concentrations of 18-20 mg/l TNT were shown to be optimum for treatability by activated sludge systems[34]. In the same study it was reported that TNT wastewater exerted a toxic effect as the concentrations were increased, as shown by a decrease observed in ultimate BOD. Greater TNT concentrations increased the F/M ratio with TNT as the available food. We have observed a co-metabolism phenomenon in our studies as readily degradable carbon sources and nutrients were present for TNT to be transformed at a rapid rate, as negligible transformation occurred utilizing TNT as the sole carbon and energy source. This was alluded to by Won as he observed more rapid transformations of TNT in a yeast extract broth versus glucose only, with TNT concentrations of 100 ppm. Enzinger[3] reported an increase in MLSS in TNT bioxidation units versus control. An increase in turbidity was also observed in pure culture studies when grown in the presence of TNT. Growth rate studies performed in our laboratories from indigenous populations isolated from IAAP and JAAP sediments exhibited a greater cell biomass as determined by absorbance data when grown in the presence of TNT as compared to the same inoculum grown without TNT. This was observed with concentrations of 10 ppm and 100 ppm TNT.

Oxygen uptake, as a parameter for measuring microbiological activity, was not shown to be affected in Nay's study, but the ability of the microorganisms to remove the carbonaceous segment of the combined wastes was affected by the TNT concentration and the detention time. Enzinger[3],

monitoring oxygen uptake in the presence of TNT, concluded that TNT retards biological activity, but near the completion of his study the difference in O_2 uptake was minimal, and at times O_2 uptake in the TNT test unit was greater than the control unit. Test units acclimated to TNT exhibited a greater O_2 uptake compared to non-acclimated cultures grown in the presence of TNT.

Data on oxygen uptake rates of indigenous microbial populations at IAAP and JAAP exhibited no adverse effects in the aqueous phase. Concentrations of TNT in the water column exceeded 1 mg/l at JAAP (Table 18). Oxygen uptake by microbial benthic communities was not retarded by the presence of TNT. The additionof TNT to select samples increased the rate of oxygen uptake. Concentrations of TNT in the sediments were generally less than 10 mg/kg (Tables 15and 21), but concentrations as high as 338 mg/kg (station T1 - JAAP) and 44,200 mg/kg (station T1A - JAAP) were observed. Bacterial cultures have been reported to transform TNT [2,3]. Pure cultures were shown to rapidly transform 100 ppm TNT to 1.25 ppm over a period of five days.[3] Enrichment cultures from indigenous sediment samples at IAAP and JAAP selected for Pseudomonas-like organisms, as determined by the characteristic greenish pigment which diffused throughout the medium. The cultures had the ability to transform 90% of the TNT added to the medium depending on the nutrient conditions.

The effect of TNT on microorganisms was recently studied by Klaushiemer [35]. A severe inhibitory effect was found on gram-positive bacteria, yeasts and fungi at concentrations of 50 mg/l TNT and greater. Preliminary disc sensitivity studies with a gram-negative bacterium, a gram positive bacterium and a yeast showed the latter two organisms to be inhibited by TNT in the growth medium. Yeasts and filamentous fungal populations observed in the aqueous phase at JAAP were somewhat reduced in numbers at stations exhibiting higher TNT concentrations as compared with the control station.

Observations on the growth of gram-negative bacteria indicated that this

group grew well at concentrations of 100 ppm and greater[35]. Results obtained confirm this hypothesis as the predominate organisms in our enrichment broths were Pseudomonas-like organisms. Studies have also indicated that gram-negative bacteria comprise over 90 percent of the indigenous bacterial flora isolated from aquatic systems[40].

The possibility exists that concentration of munitions wastes found in the sediments may be selecting for a microbial population which does not exhibit a toxic response to these compounds and is able to assimilate these nutrient availability. This population is fairly well established as shown from cell density and microbial activity studies, but may not be as diverse as populations not subjected to munitions wastes.

The metabolic fate of TNT has been examined in a number of studies. Won[2] reported that TNT is rapidly degraded and observed the appearance of "azoxy", "monoamine", "hydroxylamine", and "diamine" derivatives. The "azoxy" derivatives disappeared and the remaining products were the 2-amine-DNT and the diamines. Concentrations of the compounds were not reported by Won.

Aerobic systems, as reported by McCormick,[36] reduce TNT to the monoamine transformation product. The "hydroxylamine" intermediates may conjugate or combine with the monoamine product to form the "azoxy" derivative. Under anaerobic conditions the reduced product has been reported to be a reactive polyaniline compound.

Channon[4] formulated a series of probable metabolic proudcts which may arise when TNT is administered to animals. Reduction of the nitro groups, oxidation of the methyl constituent with the possibility of simultaneous redox reactions on the parent compound, and conjugated forms coupled with acids were suggested. Attempted mass balance determinations by Channon accounted for 5-10% of the total administered TNT as the "hydroxylamine" product and approximately 15% as the dinitroaminotoluenes. Acid conjugated products account for 50% and the remainder consists of aromatic amino compounds with a free diazotizable amino group.

The transformation products quantified in our laboratory degradation studies were tentatively identified as the 2, amino-4,6, dinitrotoluene and 4, amino - 2,6, dinitrotoluene. In both the IAAP and JAAP extracts, the 2, amino- 4,6 DNT accounted for 4-10% of the total TNT added. The 4, amino - 2,6, DNT was isolated in similar quantities from the JAAP extracts, but was present to a much lesser degree in the IAAP extracts. Semi-quantitative determinations of HPLC peaks thought to represent "azoxy" compounds suggested their presence at approximately 5% of the total TNT added. Thin layer chromatographic determinations showed "azoxy" compounds to be present when undetectable by HPLC methods. The presence of the "diamine" compound was determined by TLC methods but quantification was beyond the analytical scope of the project.

Anaerobic Digesters and Lake Sediment Microcosms

Anaerobic digesters are sensitive to environmental stresses, such as the introduction of toxic compounds. This sensitivity can be explained in terms of the microbiological populations inherent in fermentation. The initial decomposition stages of the fermentation of organic wastes are mediated by a mixed population of bacteria termed the "acid formers" that oxidize complex organic compounds to low molecular weight products. This population is not particularily sensitive to environmental stress. A second population, termed the "methane formers", reduces carbon dioxide and oxidizes the low molecular weight compounds to produce methane. The "methane formers" are sensitive to environmental stress.

The results of TNT addition to the anaerobic digesters are shown in Table 41. Digester 1 did not exhibit any chronic toxic responses, as methane production returned to a normal rate within 48 hrs. following addition of TNT, with the exception of the addition of 141.5 mg/l of TNT on 10/29, which caused a significant decrease in total gas production persisting for approximately 10 days. Digester 2 received greater amounts of TNT. Initially the addition of 164.8 mg/l TNT completely inhibited gas production indicating an acute toxic effect on the methane producing community. The digester was restarted and lower amounts of TNT were added. The concentrations were steadily increased to 201.5 mg/l, at which concentration no long term effects were observed. Increasing the concentration to 283 mg/l exerted a drastic acute effect on the digester, as gas production decreased 87% and did not return to normal.

An attempt was made to isolate and identify any transformation products which could account for the toxic effect on the microorganisms, rather than a toxic effect due to the parent compound itself. Figure 13 shows the results of thin layer chromatography plates of benzene extracted digester samples. Sample 1 was the control, sample 2 was extracted 24 hours after the addition of 165 mg/l TNT and sample 3 was extracted one month after the addition of 283 mg/l TNT.

TABLE 41 . THE EFFECT OF
TNT ON ANAEROBIC DIGESTION

	DIGESTER 1			DIGESTER 2		
DATE	INITIAL GAS PROD.(ml/l/day)*	TNT ADDED (mg/l)	%DECREASE IN GAS PROD.'	INITIAL GAS PROD.(mg/l/day)*	TNT ADDED (mg/l)	%DECREASE IN GAS PROD.'
9/19	675	74.2	40	640	164.8	100 (terminated)
9/25	-	-	-	925	122.5	25
10/9	660	82.2	58	825	122	36
10/15	625	75	8	825	125.2	6
10/22	690	100	35	850	150	35
10/29	700	141.5	75	950	201.5	45
11/22	675	128.5	52	775	283	87

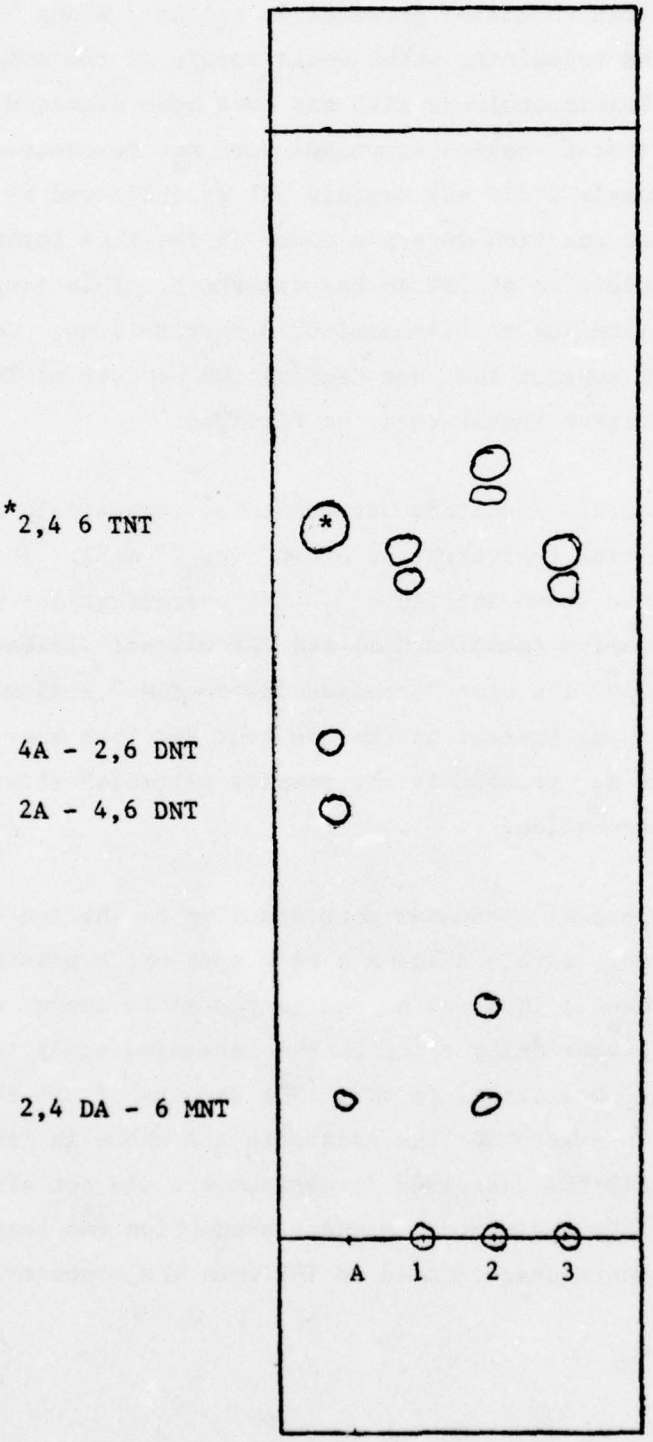
* Five day average prior to the addition of TNT

' Decrease measured 24 hours following addition of TNT

The digesters returned to a normal gas production rate within two days following the addition of TNT with the exceptions of Digester 1 on 10/29 and Digester 2 on 11/22.

Figure 13. Anaerobic Microbiological Degradation Study of TNT

Thin Layer Chromatography



Sample Key -
A - 2,4,6trinitrotoluene.
4 amino 2,6 dinitrotoluene
2 amino 4,6 dinitrotoluene
2,4 diamino 6 nitrotoluene

1 - Control, no TNT

2 - 24 hours after TNT concentration of 165 mg/l was added.

3 - One month after TNT concentration of 283 mg/l was added.

Adsorbent: Activated silica gel
Solvent: Ethyl Ether/Benzene (70/30)

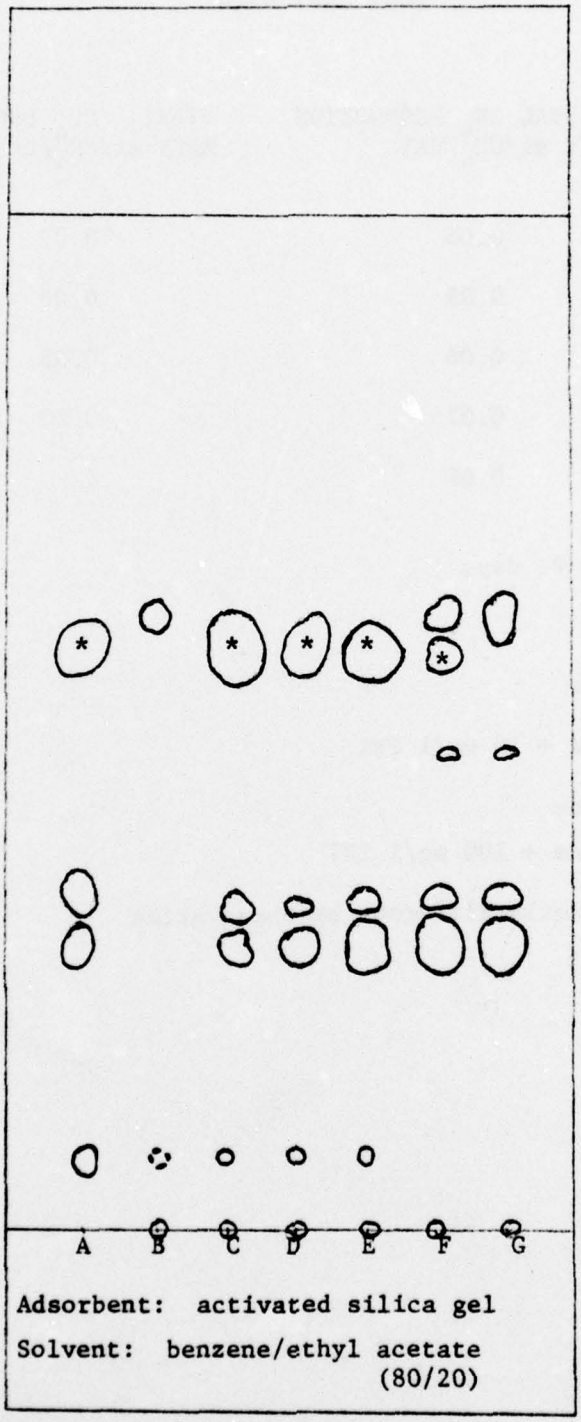
Comparisons were made of the Rf values of the unknown compounds with standards (Sample A). Sample 1 and sample 3 appear identical. This may be due to the TNT transformation products in sample 3 being highly polar compounds, such as triamines, which would remain at the origin in this solvent system. Trinitrotoluene also may have been degraded to the extent that suspected transformation compounds were not demonstratable in this TLC system. Sample 2 did not contain TNT as indicated by UV fluorescence or color reaction determinations of the thin layer plates 24 hrs. following the addition of TNT to the digesters. This sample contained a spot corresponding to 2,4-diamino, 6,nitrotoluene. Comparisons between samples 2 and 3 suggest that one degradation product of TNT is the diamine which may be further transformed or degraded.

The fate of TNT in anaerobic digesters was monitored intensively over a 48 hr. period. The initial concentration of TNT was 75 mg/1. The results of this study are shown in Figure 14. TNT concentrations were reduced over a 24 hr. period (samples C-F) and TNT was not discernable after 48 hrs. (sample G). The spot corresponding to the 2,4-diamino, 6-nitrotoluene standard was present in the one hour and four hour benzene extracts but is not present in the samples extracted after more than four hours incubation.

In this study the presence of compounds corresponding to the two monoamines were observed. Sample B shows a weak spot which possibly corresponds to the diamine. This may be due to the small amount of TNT (2 mg) added to the digester daily prior to the intensive study to acclimate the bacterial populations to TNT. The results of the effect of TNT on gas production in anaerobic lake sediments are shown in Table 42. Concentrations of 10 mg/1 TNT dissolved in cyclohexane did not affect methane production in lake sediments. Methane production was completely inhibited in lake sediments unacclimated to TNT when the concentrations were raised to 100 mg/1.

Figure 14. Anaerobic Microbiological Degradation Study of TNT

Thin Layer Chromatography



Sample Key -

A - 2,4,6 trinitrotoluene
4 Amino 2,6 dinitrotoluene
2 Amino 4,6 dinitrotoluene
2,4 diamino 6 nitrotoluene

B- Before TNT addition

C- 1 hour after TNT addition

D- 2 hours after TNT addition

E- 4 hours after TNT addition

F- 24 hours after TNT addition

G- 48 hours after TNT addition

TABLE 42. THE EFFECT OF TNT ON GAS PRODUCTION IN ANAEROBIC LAKE SEDIMENTS

SAMPLE	INITIAL CH_4 PRODUCTION RATE ml/CH_4/DAY	FINAL CH_4 PRODUCTION RATE ml/CH_4/DAY	ΔCH_4 RATE
1	0.08	0.09	N.S.
2	0.05	0.06	N.S.
3	0.06	0.05	N.S.
4	0.07	0.20	~3X
5	0.08	0	terminated

Incubation period - 21 days

1- control

2- 10 µl cyclohexane

3- 10 µl cyclohexane + 10 mg/l TNT

4- 100 µl cyclohexane

5- 100 µl cyclohexane + 100 mg/l TNT

N.S. - Not significantly different by observation

Aromatic compounds have been reported to be degraded under anaerobic conditions[37,38]. Microbiological activity under strict anaerobic conditons is generally correlated with methane production.

The digester studies showed that initial concentrations of 165 mg/l TNT will exert an acute effect on methanogenic activity. This effect was shown to decrease as the populations became acclimated to TNT. Conceivably, strict anaerobic conditions could be encountered in areas receiving munition wastes. Slug discharges accumulating in the sediments may drastically alter the ability of the bacterial populatins to degrade organic compounds and effect mineralization. The inhibition of the methanogenic bacteria could cause an increase in low molecular weight compounds which in turn could exert a toxic effect on the fermentation of available organic compounds.

The anaerobic metabolism of TNT has been ovserved by McCormich[36]. It was determined that TNT is transformed to a triaminotoluene product. Also observed were monoamine and diamine transformation products of TNT. Our studies have tentatively identified the diamine transformation product (using TLC methods) to persist during conditions of reduced methanogenic activity. Analytical techniques were not developed for detection of triaminotoluene. Information concerning the toxicity of TNT to microorganisms grown under highly reducing conditons has not been observed. The studies done with lake sediment microcosms further narrowed the range of TNT toxicity. Concentrations of 100 mg/l TNT caused a total cessation of methane production.

Gravel Percolater Microcosm

A diversified biological community exists in a gravel percolation microcosm. The surfaces provide a favorable environment for bacteria, fungi, and algae. Ciliates and other microcrustaceans are also present. The activity of the

heterotrophic, microbiological community was monitored by following acetate utilization. A fairly consistant rate of acetate utilization was obtained prior to TNT addition as shown in Table 43. Percolaters 3 and 4 were exposed to TNT for a seven day period. A decrease in acetate utilization followed. This decrease was also observed in the control percolaters. These observed decreases may have been due to a predominance of chemolithotrophic and photolithotrophic bacteria. The utilization and/or degradation of TNT was not monitored as this was beyond the scope of the project.

Disc Sensitivity Studies

Preliminary disc senstivity studies were conducted to obtain rapid reliable information on the toxicity of TNT and related compounds on a diversified group of microorganisms in an attempt to correlate laboratory toxicity studies to TNT levels encountered in receiving streams.

Table 44 indicates that TNT and one major transformation product were toxic to a gram positive bacterium, Staphylococcus aureus, and a yeast Candida albicans. These compounds however did not affect the growth of Pseudomonas aeruginosa. High counts of pseudomonad species were obtained in previous studies at the Iowa AAP [1].

A survey of the toxicity of munitions related compounds to bacteria was undertaken to obtain insight as to whether expected munition compound transformation products would detoxify the parent compound or produce a compound with a higher toxicity. The results of this survey are shown in Table 45. Toxicity was observed for three compounds on both the gram negative and gram positive bacterium. These compounds were; m-dinitrobenzene, 2,4,6-trinitrobenzaldehyde, and 3,4-toluene diamine. Azoxybenzene exerted a partial toxic effect on Staphylococcus aureus.

TABLE 43. THE EFFECT OF TNT ON GRAVEL PERCOLATER MICROCOSMS

Percolater	% Acetate Utilization Prior to TNT addition*	TNT Addition mg/l	% Acetate Utilization Following 7 day TNT Exposure
1	28.3 ± 6.5	control	10
2	56.3 ± 11.0	control	7
3	51.3 ± 11.5	53.6	11
4	42.3 ± 13.9	100.2	24

* Mean ± std. dev. (avg. of three readings)

Table 44. DISC SENSITIVITY STUDY OF TNT AND MONOHYDROXYLAMINE - DNT.

	2,4,6 - TNT zone of inhibition (mm)	Monohydroxylamine - DNT zone of inhibition (mm)
Pseudomonas aeruginosa	0	0
Candida albicans	4	2
Staphylococcus aureus	4	4

TNT - 2.5 mg per disc

monohydroxylamine - DNT - 6 mg per disc.

Table 45 . DISC SENSITIVITY STUDIES OF MUNITIONS RELATED COMPOUNDS.

Compound	Staphylococcus aureus zone of inhibition (mm)	Klebsiella sp. zone of inhibition (mm)
P-toluidine	0	0
m-toluidine	0	0
azoxybenzene	(2)*	0
2,6 DNT	0	0
0-nitrotoluene	0	0
m-dinitrobenzene	2	3
2,4,6-trinitrobenzaldehyde	10	3
2-amino 4,6-DNT	0	0
2,4 dinitroaniline+	0	0
3,4 toluene diamine	2	4
azobenzene	0	0

* parentheses indicate unclean zones of inhibition.
0.5 mg compound per disc
+ 0.25 mg compound per disc

SECTION VIII

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APPENDIX I
SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK STATION G1: 0-10 cm SECTION

Parameter	Units	Core Number G1-C	Mean	Std. Dev.
Total Solids	% dry weight	72.7	72.7	-
Total Volatile Solids	mg/kg	6.2	6.2	-
COD	mg/g	33	33	-
Hexane Extractables	mg/kg	190	190	-
Kjeldahl-N	mg/kg	1410	1410	-
Nitrate+Nitrite-N	mg/kg	300	300	-
Total Phosphorus	mg/kg	1010	1010	-
Cadmium	mg/kg	1	1	-
Chromium	mg/kg	6.2	6.2	-
Iron	mg/g	13.3	13.3	-
Mercury	mg/kg	0.06	0.06	-
Manganese	mg/kg	940	940	-
Lead	mg/kg	38	38	-

APPENDIX II
SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK STATION G2: 0-10 cm SECTION

Parameter	Units	Core Number		Mean	Std. Dev.
		G2-C	G2-Eh		
Total Solids	% dry weight	81.7	81.1	81.4	0.4
Total Volatile Solids	%	2.1	1.8	2.0	0.2
COD	mg/g	6	3	5	2
Hexane Extractables	mg/kg	170	140	160	20
Kjeldahl-N	mg/kg	360	170	270	130
Nitrate+Nitrite-N	mg/kg	110	70	90	30
Total Phosphorus	mg/kg	450	470	460	10
Cadmium	mg/kg	1	2	2	1
Chromium	mg/kg	8.4	4.4	6.4	2.8
Iron	mg/g	6.0	4.8	5.4	0.8
Mercury	mg/kg	0.04	0.04	0.04	-
Manganese	mg/kg	210	300	260	60
Lead	mg/kg	23	34	29	8

APPENDIX III
SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T1: 0-10 cm SECTION

Parameter	Units	Core Number T1-C	T1-Eh	Mean	Std. Dev.
Total Solids	% dry weight	74.3	74.2	74.3	0.1
Total Volatile Solids	%	5.4	3.8	4.6	0.6
COD	mg/g	34	39	37	2
Hexane Extractables	mg/kg	510	1090	800	410
Kjeldahl-N	mg/kg	1520	1330	1430	130
Nitrate+Nitrite-N	mg/kg	160	220	190	40
Total Phosphorus	mg/kg	820	710	770	80
Cadmium	mg/kg	1	1	1	-
Chromium	mg/kg	12.0	10.7	11.4	0.9
Iron	mg/g	9.5	8.8	9.2	0.5
Mercury	mg/kg	0.38	0.28	0.33	0.07
Manganese	mg/kg	220	210	220	10
Lead	mg/kg	158	151	155	5

APPENDIX IV
SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T1A: 0-10 cm SECTION

Parameter	Units	Core Number T1A-Eh	Mean	Std. Dev.
Total Solids	% dry weight	60.3	60.3	-
Total Volatile Solids	%	21.5	21.5	-
COD	mg/g	247	247	-
Hexane Extractables	mg/kg	4950	4950	-
Kjeldahl-N	mg/kg	9820	9820	-
Nitrate+Nitrite-N	mg/kg	260	260	-
Total Phosphorus	mg/kg	920	920	-
Cadmium	mg/kg	1	1	-
Chromium	mg/kg	14.8	14.8	-
Iron	mg/g	15.8	15.8	-
Mercury	mg/kg	0.51	0.51	-
Manganese	mg/kg	230	230	-
Lead	mg/kg	984	984	-

APPENDIX V
SEDIMENT PHASE CHEMICAL DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T2: 0-10 cm SECTION

Parameter	Units	Core Number		Mean	Std. Dev.
		T2-C	T2-Eh		
Total Solids	% dry weight	56.3	59.8	58.1	-
Total Volatile Solids	%	8.3	8.9	8.6	-
COD	mg/g	56	78	67	-
Hexane Extractables	mg/kg	750	360	560	-
Kjeldahl-N	mg/kg	1870	1780	1830	-
Nitrate+Nitrite-N	mg/kg	230	230	230	-
Total Phosphorus	mg/kg	830	830	830	-
Cadmium	mg/kg	2	1	2	-
Chromium	mg/kg	8.3	9.4	8.9	-
Iron	mg/g	13.5	14.7	14.1	-
Mercury	mg/kg	0.25	0.18	0.22	-
Manganese	mg/kg	320	670	500	-
Lead	mg/kg	93	85	89	-

APPENDIX VI
SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK STATION G1: 0-10 cm SECTION

Parameter	Units	Core Number G1-C	Mean	Std. Dev.
2,6-Dinitrotoluene	mg/kg	< 1	< 1	-
2,4-Dinitrotoluene	mg/kg	< 1	< 1	
1,3,5-Trinitrobenzene	mg/kg	< 1	< 1	-
2,4,6-Trinitrotoluene	mg/kg	< 1	< 1	-
4-Hydroxylamino-2,6-Dinitrotoluene	mg/kg	< 5	< 5	-
2-Hydroxylamino-4,6-Dinitrotoluene	mg/kg	<30	< 30	-

APPENDIX VII
SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
GRANT CREEK STATION G2: 0-10 cm SECTION

Parameter	Units	Core Number G2-C	Core Number G2-Eh	Mean	Std. Dev.
2,6-Dinitrotoluene	mg/kg	<1	<1	<1	-
2,4-Dinitrotoluene	mg/kg	<1	<1	<1	-
1,3,5-Trinitrobenzene	mg/kg	<1	<1	<1	-
2,4,6-Trinitrotoluene	mg/kg	1	<1	1	-
4-Hydroxylamino-2,6-Dinitrotoluene	mg/kg	<5	<5	<5	-
2-Hydroxylamino-4,6-Dintrotoluene	mg/kg	<30	<30	<30	-

APPENDIX VII
SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T1: 0-10 cm SECTION

Parameter	Units	Core Number		Mean	Std. Dev.
		T1-C	T1-Eh		
2,6-Dinitrotoluene	mg/kg	2	204	103	142
2,4-Dinitrotoluene	mg/kg	12	675	344	469
1,3,5-Trinitrobenzene	mg/kg	<1	<1	<1	-
2,4,6-Trinitrotoluene	mg/kg	40	635	338	421
4-Hydroxylamino-2,6-Dintrotoluene	mg/kg	24	47	36	16
2-Hydroxylamino-4,6-Dintrotoluene	mg/kg	63	97	80	24

APPENDIX IX
SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T1A: 0-10 cm SECTION

Parameter	Units	Core Number T1A-Eh	Mean	Std. Dev.
2,6-Dinitrotoluene	mg/kg	6	6	-
2,4-Dinitrotoluene	mg/kg	642	642	-
1,3,5-Trinitrobenzene	mg/kg	3	3	-
2,4,6-Trinitrotoluene	mg/kg	44200	44200	-
4-Hydroxylamino-2,6-Dintrotoluene	mg/kg	154	154	-
2-Hydroxylamino-4,6-Dintrotoluene	mg/kg	140	140	-

APPENDIX X
SEDIMENT PHASE MUNITIONS DATA
JOLIET ARMY AMMUNITION PLANT 3 JUNE 1975
TNT DITCH STATION T2: 0-10 cm SECTION

Parameter	Units	Core Number T2-C	Core Number T2-Eh	Mean	Std. Dev.
2,6-Dinitrotoluene	mg/kg	< 1	< 1	< 1	-
2,4-Dinitrotoluene	mg/kg	< 1	< 1	< 1	-
1,3,5-Trinitrobenzene	mg/kg	< 1	< 1	< 1	-
2,4,6-Trinitrotoluene	mg/kg	5	1	3	3
4-Hydroxylamino-2,6-Dinitrotoluene	mg/kg	15	< 5	10	7
2-Hydroxylamino-4,6-Dinitrotoluene	mg/kg	< 30	< 30	< 30	-

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